ELLIPTOCYTE TRANSFUSIONS

AS A METHOD IN STUDIES ON BLOOD DESTRUCTION, BLOOD VOLUME, AND PERITONEAL RESORPTION

BY

STURE HEDENSTEDT

This Work is also published as Supplementum 128 of Acta Chirurgica Scandinavica

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ALMQVIST & WIKSELLS BOKTRYCKERI AB

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1/48

FROM THE PHYSIOLOGICAL DEPARTMENT (HEAD: PROFESSOR T. TEORELL) AND THE SURGICAL CLINIC (HEAD: PROFESSOR O. HULTÉN) OF THE UNIVERSITY OF UPPSALA, SWEDEN.

To my Father

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Introduction and Preface.

Since the autumn of 1944 the author has been engaged in working out *a method whereby elliptocytes may be used as labelled corpuscles* (*"the elliptocyte method"*). The work was started as a result of the author coming across a case of the anomaly in Karlstad. Systematic search later led to the discovery of more cases. It is obvious that elliptocytic blood can be used for the solution of many different problems, both physiological and pathological. The most important part of this work is devoted to transfusion experiments and investigations into the resorption of blood from the abdominal cavity. Methods using artificially produced radioactive isotopes have recently come into frequent use. The author therefore carried out experiments using such methods, primarily in order to check the results obtained by the use of elliptocytes.

The work included an attempt to determine the *lifetime of red blood corpuscles* after transfusion, attempts to determine the *blood corpuscle volume* and *blood volume*, and finally experiments on the *resorption of blood from the abdominal cavity*. These last experiments are of particular interest from a surgical point of view.

As previously mentioned, while a member of the staff of the hospital at Karlstad, I began the present investigations. My former chief, Dr. Sven Örström aided me greatly by making it possible for me to be free to carry on my investigations, for which I wish to convey my warm thanks.

Later when I joined the staff of the Surgical Clinic of the University Hospital in Uppsala, my present chief, Professor Olle Hultén extended to me the same courtesy. For his interest and helpfulness I am deeply grateful.

In my investigations I have had the privilege of examining patients from the Child Clinic of the University Hospital and wish to thank Professor Curt Gyllenswärd for this courtesy.

The main experimental work has been carried on at the Physiological Institute of Uppsala University. I wish to thank the head

of the Institute, Professor Torsten Teorell for the privilege of working at the Institute and for his cooperation and interest, and especially for the help he has given me in the problem of transperitoneal resorption.

My thanks go also to Professor Robin Fåhræus for the interest he has shown in my work and the expert advise on questions of blood physiology.

I wish to thank Associate Professor Henrik Enghoff who has so kindly placed instruments at my disposal, and discussed my work with me.

In order to carry out my investigations with radioactive substances, Docent Åke Örström of the Wenner-Gren Institute in Stockholm has rendered valuable assistance. I wish to express my great indebtedness to him.

I wish to thank Associate Professor Nils Gellerstedt who has helped me with the histological investigations.

The statistical working up of my material has to a great extent been carried on by the State Institute of Race Biology at Uppsala. I wish to thank Professor Gunnar Dahlberg for the valuable help he has given me. Also the staff of the Institute, especially Actuary Elvir Lander for his assistance.

Furthermore I wish to thank all who in the giving of their blood have made it possible to carry on this investigation — quite especially Miss Gunborg Malm of the nursing staff of Karlstad on whom the heaviest burden has been placed.

Miss Olga Andersson of the nursing staff of Uppsala who by sacrificing her leisure time, assisted me in making blood transfusions; Miss Ulla Eklind and Miss Lisbeth Pettersson who as my assistants with untiring interest have helped me in taking blood counts — I wish to thank them heartily. To Miss Doris Fransson who with the utmost conscientiousness has taken part in many of the investigations and to my sister Mrs. Kerstin Kjellgren who has put the microscopic sections in order — I owe a deep debt of gratitude.

For her rapid and conscientious translation my sincere thanks go to Dr. Ann Synge.

I have received financial assistance for my investigations from the Swedish Society for Medical Research, from the Regnell Fund and a Government grant for Medical Research through the courtesy of the Medical Faculty.

Furthermore a scholarship from the Svea Andersson Memorial Fund was given to me through the courtesy of the society of Younger Swedish Physicians.

To my father, formerly Lektor, Dr. Birger Hedenstedt, who has always been my example, I wish to direct my warmest thanks. With never ending interest and indefatigable energy he has worked up a great part of the material dealing with mathematical calculations.

Uppsala — March 1947.

Sture Hedenstedt.

Part I. Review of the Literature.

FIRST CHAPTER.

Elliptocytosis (ovalocytosis).

Elliptocytosis (ovalocytosis) was first described by Dresbach (1904). His case was that of a mulatto student 90 % of whose red blood corpuscles were stated to have been elliptical. He was otherwise healthy.

Many cases have been published since then. Wyandt et al. (1941) collected the cases which had been published up to that time and themselves contributed the largest series consisting of 86 elliptocytics. According to them at least 330 cases of elliptocytosis (ovalocytosis) were known up to that date. One may assume that the condition is rare since it was not described earlier in spite of the large number of blood investigations which had been carried out. The first investigation into the frequency of the condition was carried out by McCarthy (1934). He found 4 cases of the anomaly amongst 10 000 persons investigated. Wyandt et al. found 2 cases of the anomaly in 7 000 blood investigations.

Character of the anomaly.

The concept of ovalocytosis and ovalocytes was introduced into medical literature by Hijman van den Bergh (1928) and it was defined as an anomaly in which the red blood corpuscles were to a large extent elliptical. Most of his cases displayed no particularities apart from the characteristic shape of the red blood corpuscles.

It has been stressed by several authors, for example Söderström (1938), Lambrecht (1938), Wyandt et al., that the expressions ovalocytosis, ovalocytes etc. are misleading as the characteristic shape of the corpuscles is geometrically more like an ellipse and is not oval i. e. egg-shaped. Lambrecht proposes that the terms

elliptocytosis and elliptocyte should be used instead and there is no doubt that these expressions are more suitable. As the authors of the most recent literature show a general tendency to change over to the terms elliptocytosis and elliptocyte it seems to the present author to be completely justifiable to give up the terms ovalocytosis and ovalocyte altogether. The expressions *elliptocytosis*, *elliptocyte* and *elliptocytic* will therefore be used in connection with the condition in which most of the red blood corpuscles correspond morphologically to the description given below, regardless of the clinical characteristics of the case in other respects.

Söderström gives a summary of the morphological characteristics of the elliptocyte. Elliptocytes are red blood corpuscles of more or less markedly elliptical shape. All intermediate forms exist between the perfectly round red blood cells which are never completely absent, and very elongated elliptical forms in which the longest diameter may considerably exceed twice the diameter of a normal erythrocyte. The longer the cell the thinner it is. Elliptocytes are as a rule biconcave, only the more elongated forms may sometimes lose their central depressions and assume a more rounded form.

It is stated by Dresbach and in most of the later publications that it is usual for 70– 90 % of the blood corpuscles to be elliptical in cases of this anomaly. Günther (1928) was the first to attempt to derive more certain information as to the morphology of the erythrocytes in a systematic way by means of moasurements of their diameters. He was able to determine the degree of elliptocytosis or the numerical eccentricity according to the formula $e = \sqrt{1 - \left(\frac{b}{a}\right)^2}$ by means of measurements of the long (a) and short (b) diameters of a large number of erythrocytes. It was shown by this method that the extreme of eccentricity of the corpuscles in normal people varied between $e = 0.08$ and 0.71 with a mean value of 0.46 and a standard deviation of 0.16. The number of cells measured was 200. It is easily seen from the formula that in round blood corpuscles $e = 0$ and that as the value of e increases the elliptical shape becomes more and more pronounced. For practical clinical work Günther used a simpler procedure which he calls "intuitive estimation", as he divides

[1] The eccentricity is usually calculated from the formula $e = \frac{c}{a}$ where $c = {}^1/_2$ the distance between the foci and $a = {}^1/_2$ long axis.

the erythrocytes into 4 classes under the microscope by eye. He proceeded on the assumption that when examining a blood film, it was possible to differentiate by eye between I circular, II roundish, III elliptical and IV elongated elliptical erythrocytes. By intuitive estimation of 300—400 cells at a time he was able to place the corpuscles in the 4 classes and express the frequency of the different forms in percentages.

A comparison of the results of estimation with those of exact measurement showed that the intuitive estimator was liable to estimate as circular cells with a slight degree of eccentricity, up to 0.47. The shape was first stated to be elliptical when the eccentricity was about 0.62 and when it was more than 0.74 the shape was described as markedly elliptical. Günther states that one must expect a mean error of 2—3 % in estimation. Lambrecht has made a collection of the normal values for the eccentricity found by various authors using Günther's method, table 1.

Table 1. Degree of ellipticity in normal cases according to different authors.

Class I (Circular)	Class II (Roundish)	Class III (Elliptical)	Class IV (Elongated elliptical)	Class III + IV	Author
0.0—0.47	0.47—0.62	0.62—0.74	> 0.74	> 0.62	
74.4	14.9	8.2	2.5	10.7	Günther (1928)
51.7	38.1	10.0	0.2	10.2	Penati (1930)
72.79	14.45	8.49	1.09	9.58	Lambrecht (1938)

If Günther's classes I and II are taken together and considered as round and classes III and IV are taken together and considered as elliptical it is found from the investigations of Günther, Penati (1930) and Lambrecht, which were in agreement, that normal blood contains about 10 % of elliptocytes. Lambrecht suggests that, from a practical point of view, the diagnosis of elliptocytosis should not be made unless the total elliptocyte count, i. e. the sum of Günther's classes III and IV exceeds 25 % of the red blood cell count.

In cases of anaemia, specially those of the pernicious type, the elliptocyte count may exceed 10 %. However, in these cases the short forms predominate (Class III) while in elliptocytosis the elongated

forms (class IV) are relatively common. This information is derived from Leitner's review of 1939, table 2.

Table 2. Degree of ellipticity in different diseases.

Class I (Circular)	Class II (Roundish)	Class III (Elliptical)	Class IV (Elongated elliptical)	Author
0.0—0.47	0.47—0.62	0.62—0.64	> 0.74	
51.7	38.1	10.0	0.2	Normal cases Penati (1930)
32.94	44.94	20.04	2.53	Secondary anaemia Penati(1930)
17.4	48.2	32.4	2.0	Pernicious anaemia Penati(1930)
1—7.5	17.5—33.5	37—39.5	22.0—42.0	Elliptocytosis Leitner (1939)

Günther's classification has been used by many authors e. g. Penati, Grzegorzewski (1933), Söderström, Lambrecht and Leitner. Most of the earlier authors did not divide the elliptocytes into classes but gave the elliptocyte count as obtained directly from a differential count. Even some more recent authors such as Wyandt et al. determined the elliptocyte count without classification and considered as elliptical all corpuscles in which there was a definite difference between the long and the short diameters. There is a form of elliptocytosis which occurs among the relatives of elliptocytics. These are called latent elliptocytics (Lambrecht) or partial elliptocytics (Leitner), and are distinguished by a considerably lower elliptocyte count than ordinary elliptocytics. However, according to Lambrecht these cases exhibit more than 25 % of short and elongated elliptocytes (Classes III and IV).

Constancy of the anomaly.

As early as 1904 Dresbach established that his case had a constant elliptocyte count over a period of 4 months which was as long as he followed him. Bishop (1914) followed a case over a period of 8 months and arrived at the same result. It has also been confirmed by later writers, for example Huck and Bigalow (1923), Leitner and Wyandt et al. by means of repeated observations on the same individual over longer periods. Günther described a case which showed a more marked elliptocytosis when anaemic than when not

anaemic. Söderström described a similar case. In Günther's case, however, the diagnosis of elliptocytosis was open to question as there were several points indicative of pernicious anaemia, which might be the explanation of the high percentage of elliptical cells, for, as is seen from table 2, this may occur in pernicious anaemia. In Söderström's case the elliptocyte count was not so markedly raised; he describes a regression from 18 %—4.1 % of cells in Günther's class IV during about 5 months. It may be that this was a case of pronounced poikilocytosis associated with a hypochromic anaemia. Pollock and Dameshek (1934) found daily variations which may be due to their having confused elliptocytosis with the picture characteristic of drepanocytosis as they definitely describe cases in which both elliptocytes and sickle-shaped cells occur in the same individual. However, drepanocytosis is thought to develop to a great extent in the preparation after withdrawal of the blood from the veins and to reach a maximum in 24 hours, after which the proportion of sickle-shaped cells diminishes over a period of some days (Vaubel (1937)). This should therefore clearly differentiate it from elliptocytosis.

In this connection 2 cases of elliptocytosis in the new-born should be mentioned. One was described by Hunter (1932) and the other by Wyandt et al. The first child had only a few elliptical cells in the blood from the cord at the time of birth. 3 months later the elliptocyte count was given as 41.4 % oval and 1.6 % elongated. The second case had 11 % of elliptocytes at birth and 80 % 4 months later. This suggests that the anomaly is not always developed by the time of birth.

Cell volume and haemoglobin content.

Wyandt et al. show in their review that the majority of workers in this field have found a normal cell volume. They themselves found 3 cases of slight microcytosis among 14 cases examined. Söderström found a lowered index of volume in 2 cases. Lieberherr (1938), Stephens and Tatelbaum (1935) and Leitner also describe cases with microcytosis. The cell volume is usually determined by means of the haematocrit.

In trying to form an opinion on those cases of elliptocytosis in the literature which were said also to be suffering from anaemia, one is hampered by the difficulty that most of the authors do not state what

method or haemoglobin standard they used. Kirkegaard and Larsen (1942) have considered all elliptocytics having a haemoglobin level of less than 80 % of normal and an erythrocyte count of less than 4 millions per mm^3 as anaemic without considering what methods were used. In view of what has been said above the figures are very unreliable. According to these criteria 31 out of 246 elliptocytics had an anemia without any other demonstrable cause. Half of these cases appeared to be perfectly healthy at the time of the investigation. Schartum Hansen (1935) and Söderström state that elliptocytes are paler than round cells when stained by Giemsa's method. The latter author also points out that the colour index is usually less than 1.

Effect of external circumstances on the anomaly.

It has been established by almost all the authors on this subject (e. g. Leitner) that elliptocytes show up very clearly in a fresh preparation and retain their shape very well. Günther, Leitner, Gerrits and de Vries (1942) and others have observed that fewer elliptocytes are found in dry blood-films than in fresh wet preparations under a coverslip. Leitner and other authors have established that elliptocytes retain their shape well after having been kept in an ice-chest so long as no evaporation of water takes place. Elliptocytes lose their biconcave form and become spherical to the same extent as ordinary red blood corpuscles when they are suspended in isotonic saline for a time. If however a preparation of such cells under a coverslip is allowed to stand for a time the cells gradually differentiate themselves again so that round and elliptical forms can be distinguished once more (Terry et al. (1932)). Elliptocytes consistently retain their shape in hypertonic solutions (Bernhardt (1928), Leitner).

McCarthy found that if elliptocytes were treated with lysolecithin they all became spherical, whereas cholesterol had no such effect. According to Hunter and Adams (1929) carbon dioxide had no effect on the shape of elliptocytes. Rosenow (1933) observed an increase in the number of elongated elliptocytes in the capillary blood after stasis. The same experiment was made by Leitner but he was unable to confirm the result of the previous author.

All authors are agreed that the properties of the serum play no

part in determining elliptocytosis. Leitner and a large number of other authors have mixed washed elliptocytes with normal serum and washed normal corpuscles with serum from an elliptocytic without finding any influence of the serum on the shape of the corpuscles. Neither acidification nor the addition of carbon dioxide had any effect on the shape of the elliptocytes.

Investigation of the osmotic resistance.

Investigations of the osmotic resistance are important in distinguishing elliptocytosis from the haemolytic anaemias, specially haemolytic jaundice which is said by certain authors (see below) to coexist with elliptocytosis in some cases.

In most cases of elliptocytosis (Bernhardt, Hijman van den Bergh, Cheney (1932), Roth and Jung (1931), Huck and Bigalow, Hunter and Adams (1929), Leitner, Pollock and Dameshek, Rosenow, Rotter (1933), McCarthy, Sydenstricker (1923), Söderström, Vischer (1938) and others) the osmotic resistance of the corpuscles in hypotonic saline has been shown to be normal. Evaluation of the results is, however, somewhat uncertain as the criteria on which the readings were based are not always given.

Grzegorzewski found a definite lowering of osmotic resistance in 5 cases, in that the concentrations at which haemolysis first appeared and at which it was complete were 0.66—0.40 % Na Cl, 0.60—0.40 %, 0.66 0.45 %, 0.66 0.42 % and 0.60—0.52 % respectively. Lambrecht found a slight lowering of osmotic resistance in 3 cases where the concentrations in question were 0.52—0.38 %, 0.58—0.42 % and 0.50 0.38 % Na Cl. There are further described individual cases of a similar kind not included here. Among 14 cases of elliptocytosis Wyandt et al. found one case with a markedly lowered osmotic resistance (0.85 0.20 %) and 2 cases in which the lowering was less marked (0.50—0.34 % in each case).

Terry et al. seem to have been the only authors to have succeeded in getting the elliptical corpuscles into a lower layer and the round ones into an upper layer by means of prolonged centrifugation. Tests of osmotic resistance on the upper layer gave values of 0.46 – 0.32 % Na Cl and on the lower layer (elliptocytes) 0.42 –0.24 % Na Cl while normal blood treated in the same way gave the same values for the upper and lower layers. Other authors, e. g. Leitner, Kirke-

gaard and Larsen and others, have been unable to isolate the elliptocytes in this way.

Some authors, e. g. McCarthy, Roth and Jung (1931) and others, have claimed to have found a greater degree of osmotic resistance than normal in isolated cases.

Vischer has tried to make another contribution to the study of the osmotic resistance of elliptocytes by transfusing elliptocytic blood into 5 persons with normal blood-pictures (see p. 25 and 33). He considered that he was able to show by this means that the lifetime of the elliptocytes, which he found to be on an average 13 days, was considerably shorter than that of normal blood corpuscles. He considered that this was indicative of lowered osmotic resistance in elliptocytes. Huck and Bigalow, who carried out a similar experiment, observed that no elliptocytes were to be found in the recipient's blood after 2 months (see p. 25 and 32). Kirkegaard and Larsen also carried out several transfusions of elliptocytes and found that elliptocytes were to be observed in the recipient's blood 33—36 days (see p. 25 and 33) after the transfusion. This agreed with their own attempts and those of Sköld (1943) to determine the lifetime of normal red blood corpuscles by the transfusion of normal blood into an elliptocytic (see p. 26 and 33).

By making differential counts of the blood corpuscles in the sediments in the test-tubes used in testing the osmotic resistance Söderström found that the proportion of elliptocytes in the last tube in which haemolysis was incomplete was 30—50 % higher than that in the first tube in which haemolysis occurred. Kirkegaard and Larsen achieved the same results in similar experiments. This would appear to indicate a raised osmotic resistance.

Kirkegaard and Larsen have made experiments to find out the resistance of elliptocytes under the influence of heat but were not able to exclude the possiblility that the resistance of elliptocytes to the effects of heat was less than of normal blood corpuscles under the same conditions.

The last mentioned authors have made further experiments to solve the important problem of the resistance of elliptocytes. They start with the conception that the spherical shape of the corpuscles in haemolytic jaundice is one of the causes or the only cause of the decreased resistance, as a spherical corpuscle can scarcely increase its volume without increasing its surface and this cannot be en-

larged. When a spherical blood corpuscle is unable to increase its volume further for this reason it bursts. By calculations of the volume and surface of spherocytes, normal erythrocytes, and elliptocytes the result arrived at is that they can increase their volume by 37 %, 59 % and 66 % respectively before they become completely spherical and are therefore no longer able to increase their volume. From what has just been said elliptocytes would rather be expected to show increased resistance in comparison with the osmotic resistance of normal blood corpuscles.

Reticulocytes and the bone marrow.

Relatively few authors have carried out reticulocyte counts on elliptocytic blood. According to Kirkegaard and Larsen the reticulocyte count has only been established in 21 cases apart from cases complicated by other illnesses. In 15 of these cases the patients were not anaemic and in 10 of these the proportion of reticulocytes was higher than normal. Schartum –Hansen (1935), Leitner and others have observed that the reticulocytes are usually round. According to Leitner 4 5 of the reticulocytes in elliptocytic blood are round. Wyandt et al. made reticulocyte counts in 14 cases of elliptocytosis. In one case there was a definite anaemia with a reticulocyte count of 13 %, in the rest of the cases the reticulocyte count varied between 1.5– 3.6 %. In the cases where the reticulocyte count was highest there was slight anaemia.

As the manner of development of elliptocytes in the bone marrow is of interest (i. e. the question as to whether the nucleated corpuscles are elliptical) several authors have performed sternal puncture. Bernhardt seems to have been the first to show by puncture of the marrow that the normoblasts are, without exception, round in these cases. This has moreover been verified by all such later authors as have concerned themselves with the matter. Schulten (1939) is of the opinion that the elliptocytes which are sometimes found in the material obtained by sternal puncture arise to a greater or less extent from contamination by blood at sampling which is in most cases unavoidable. Roth and Jung also found both round normoblasts and elliptocytes. Most of the authors who performed sternal puncture found a normal picture. Cheney, Lawrence (1931), Leitner, Roth and Jung, Schartum –Hansen, Söderström, Wyandt et al. and Vischer

as well as certain other writers, have stated that they found increased erythropoiesis. The number of normoblasts was increased according to Vischer, and mitoses were evident in 5 cases which were not anaemic. In Bernhardt's case there was an anaemia complicating the picture. This was caused by a septic process associated with fever and increased erythropoiesis. In other cases there was also an increased erythropoiesis associated with anaemia (Lambrecht, Junk (1938)).

White blood cells, sedimentation rate etc.

Leitner carried out differential white cell counts on 5 cases of elliptocytosis and was unable to find any peculiarities in these counts which could be associated with elliptocytosis. The sedimentation rate was also normal in those cases where it was investigated. Finally Söderström states that elliptocytes show the same tendency to rouleau formation as ordinary corpuscles and that there is nothing noteworthy about their agglutination reactions. The four blood groups are all to be found in elliptocytics.

Elliptocytics and disease.

In the majority of the cases of elliptocytosis described in the literature, the characteristic shape of the red blood corpuscles was the only symptom of interest shown by the elliptocytic, and most authors are inclined to consider elliptocytosis as a characteristic anomaly but one without further significance and occurring in completely healthy people. Wyandt et al. observed the largest series consisting of 86 new cases. They point out that the various members of the families concerned were all healthy and long-lived.

In many cases some illness exists which one is tempted to associate with the elliptocytosis. It is however, by the nature of things, unlikely, that such elliptocytics as are described by doctors will be healthy. Healthy people do not often consult a doctor. Obviously elliptocytics are no more proof against illness than people with normal erythrocytes. When the illnesses from which the elliptocytics suffer are of a temporary nature there are no grounds for suspecting any connection as the elliptocytosis remains constant. On the other hand some connection may be suspected when the illness is of a more chronic character. By the nature of the case blood diseases are the first to fall

under suspicion. In such a situation the available information must be the subject of careful criticism.

Grzegorzewski has observed jaundice in cases of elliptocytosis. He found 14 elliptocytics who were related to one another and 6 of these cases showed mild symptoms of haemolytic jaundice. In these cases the osmotic resistance was also lowered (see above). In the other 8 cases no such symptoms were present. Pensfold and Lipscomb (1943) record slight symptoms of jaundice in 12 % of their cases.

Van den Bergh had a patient with elliptocytosis, anaemia, jaundice and splenomegaly but with normal resistance. Splenectomy caused regression of the jaundice but the elliptocytosis persisted. Lambrecht also had one case with jaundice, slight anaemia, lowered resistance and raised reticulocyte count.

Bernhardt's case is doubtful as a septic condition was present which might explain the jaundice, anaemia and splenomegaly. From the description it is possible to imagine that Roth and Jung's case was one of cholelithiasis with jaundice.

Only a few authors describe splenomegaly. The estimation of splenomegaly by palpation must be considered of doubtful value. Hijman van den Bergh, Bertelsen (1938), Lambrecht and Wyandt et al. have described elliptocytics with enlarged spleens (5 cases). Bernhardt's case is doubtful (see above).

According to the point made in a previous chapter a certain proportion of elliptocytics may be expected to be anaemic. Kirkegaard and Larsen have estimated the frequency of anaemia to about 12 – 15 %. They took a Hb-value of 80 % as the upper limit for anaemia and only counted as anaemic those cases where no other cause for the anaemia could be found. About half of these would have described themselves as completely healthy at the time of the examination. The series of Wyandt et al. which is not included in the previous calculations, only contains one case of anaemia. Various writers have used the term "ovalocytic anaemia", which seems inappropriate as anaemia is not an essential concomitant of elliptocytosis but, on the contrary, according to the literature of the last few years it can seldom be demonstrated in such cases. (Wyandt et al., Gigon (1938), Leitner, Kirkegaard and Larsen). The poikilocytosis arising in connection with secondary anaemias may give a false impression of similarity.

Some of the cases of anaemia among elliptocytics have nevertheless presented symptoms which resembled those of haemolytic jaundice

to a greater or less degree. The cases which have had this symptom fully developed in combination with elliptocytosis are very few in number. Wyandt et al. had one case with all the classical symptoms of haemolytic jaundice among 86 elliptocytics. Pensfold and Lipscomb's figure of 12 % for cases with a slight degree of icterus seems rather exaggerated.

The fact that elliptocytosis and haemolytic jaundice can exist in the same individual or the same family is not of course in itself remarkable. It should be expected that two hereditary characteristics, even though rare would have a certain frequency of coincidence. The question is whether the two diseases are connected with one another in such a way that they have, in part, the same genetic background. There is no reason to believe this to be the case. Certain authors, e. g. Kirkegaard and Larsen, Schulten, Gänslen (1940), and others however include elliptocytosis as the least characteristic and mildest form of haemolytic anaemia in the same way as drepanocytosis. On the basis of a critical examination of the literature, and with special regard to the large series of Wyandt et al. which is not quoted by the other authors, *it is impossible to maintain that the earlier literature gives any definite evidence of a connection between haemolytic jaundice and elliptocytosis.*[1]

Elliptocytosis from a hereditary point of view.

As the majority of elliptocytes do not take up reticulocyte stain and as their precursors, the normoblasts, are round, most authors conclude that the elliptocytes assume their abnormal shape at the time when they change from reticulocytes to mature erythrocytes. It follows from the experiments in which the elliptocytes were suspended in various sera that the question does not involve the properties of the serum. It is thus agreed that the elliptical shape of the erythrocytes must be dependent on some property residing in the corpuscles themselves. By means of thorough investigations of resistance to haemolysis and by allowing other influences to affect the elliptocytes Kirkegaard and Larsen have come to the definite conclusion that the shape of the elliptocytes depends on forces inherent in them and is not determined by the effects of external influences. The nature of these forces is unknown.

[1] Note in the proof-sheet: When the present work was in the press Holst-Larsen (1947) published a paper treating of the subject above.

To judge by the earlier literature (Huck and Bigalow, Bishop (1941) and others) elliptocytosis is undoubtedly to be regarded as an hereditary anomaly. Gänsslen confirms this conclusion and states that a single dominant not sex-linked gene is concerned. The large pedigrees which have been published by various authors, of which that published by Wyandt et al. is the largest, beautifully demonstrate the dominant hereditary nature of the anomaly.

SECOND CHAPTER.

Use of Elliptocytes as Labelled Blood Corpuscles for Experimental Purposes.

Judging from the literature, the idea of using elliptocytes as labelled blood corpuscles for experimental purposes seems only to have been employed in a few isolated investigations.

Huck and Bigalow (1923) made an intravenous transfusion from an elliptocytic into a normal person. The elliptocytic was said to have an elliptocyte count of 53—84 % and was healthy. However, the authors only state that elliptical corpuscles were no longer to be found in the recipient's blood after 2 months, no information was given as to the elliptocyte counts in the intervening period.

In 1938 Vischer carried out 5 intravenous transfusions into patients who were in hospital but showed a normal blood picture. The counts of elliptical blood cells in each donor and recipient were determined before transfusion. The elliptocytes were counted in a blood film. The proportion of elliptocytes in the five experimental subjects varied from 0.5 – 2.5 %. The elliptocyte count in the donors varied from 85 – 90 %. The amount of blood transfused was 250 – 300 cc. After transfusion the elliptocytes were counted daily in films and in this way their concentration was determined. The time at which the elliptocyte count reached the same level as before the transfusion was noted. The time in days before they disappeared was taken as a measure of the lifetime of the elliptocytes (see also p. 33).

In 1942 Kirkegaard and Larsen published some experiments with elliptocyte transfusions which aimed at establishing the lifetime of the red blood corpuscles. Before the transfusion the elliptocytes in the recipient's blood were classified according to their shape into 4

groups by the method of Günther. In all of the experiments 500 cc of elliptocytic blood was transfused into an adult of the same group and the condition of the recipient's blood was determined every other or every third day in the same way as before transfusion. The counts were carried out in a counting chamber using blood from the ear diluted with Hayem's solution as for ordinary erythrocyte counts. Before the transfusion 10 000—20 000 cells were differentially counted and in the tests after transfusion 1 000—5 000. Certain sources of error are pointed out. One of the most important is the difficulty in estimating the shape of the erythrocytes. There are, of course, all possible intermediate forms, and opinion can vary from day to day. These authors like the previous ones have probably counted all cells which were not absolutely round as elliptocytes. For these reasons the elliptocyte count in normal blood has been found to be relatively high. In one of the cases the authors made many daily counts of smaller numbers of cells instead of a single count of a larger number. This gave a much more regular curve. By means of three transfusions of elliptocytic blood into persons having normal blood the authors have tried to determine the lifetime of the red blood corpuscles. A quite new method of approach was introduced, namely that normal blood was transfused into an elliptocytic instead, and the number of round cells was determined in the elliptocytic. No calculations of the amount of the experimental error are given. The reader is referred to p. 33 for the results of these investigations.

Sköld (1943) also transfused normal blood into an elliptocytic and calculated the count of round cells in the recipient from a blood film in the same way as the previous authors. The present author, who himself used the same elliptocytic as Sköld for many experiments, has convinced himself by careful measurements that this elliptocytic has not got 100 % elliptocytes in his blood as is stated. However, Sköld did not carry out any very detailed calculations. He finds a smaller proportion of round blood corpuscles in the recipient's blood after transfusion than would have been expected on theoretical grounds. This may be explained by a certain degree of elliptocytosis in the donor. One can also postulate that "part of the blood was withdrawn from circulation in the blood depots".

In 1945 Hedenstedt described experiments in which elliptocytic blood was introduced intraperitoneally in order to study the resorption of blood corpuscles from the peritoneal cavity, "the trans-

peritoneal resorption of blood corpuscles". In this work the author used a method of measurement similar to that employed in Günther's original experiments employing the formula $\sqrt{1 - \left(\frac{b}{a}\right)^2}$. If one only counts as elliptocytes those corpuscles which show such a marked deviation from roundness that they could scarcely be present in normal blood this method is fairly good. However, no calculations of the experimental error were made. The present author pointed out in the same work that he had also made a start with investigations of the passage of blood corpuscles through the placenta from mother to foetus using the same method (cf. p. 33).

In 1946 Hedenstedt and Naeslund showed, by means of a transfusion of elliptocytic blood into a mother ante partum in a case of nephropathia gravidarum that the elliptocytes passed from the mother into the foetus via the placenta which was much changed by so-called "infarct formation".

THIRD CHAPTER.

Destruction of the Red Blood Corpuscles.

Schiodt (1938) published a review of the work in this field up to that date. He put forward two theories concerning the concept of the lifetime of the red blood corpuscle. He called them the "theory of longevity" and the "theory of destruction".

1. *The theory of longevity.* According to this theory the corpuscles have a definite lifetime which is an innate characteristic. The organ which disposes of the corpuscles after their death acts in a purely passive way. The number of corpuscles which is destroyed in each day comprises those which have completed their lifetime during that day. Thus, every day a certain number of erythrocytes reach the end of their lifetime, die, and are removed from the circulation; but as the number of erythrocytes in any individual may be assumed to be constant, an exactly equal number of erythrocytes must be produced to replace those which die. (Individual variability has been determined by Dahlberg (1929) and was found to be comparatively slight.) One may therefore assume that, under normal conditions, there is an equilibrium between production and destruction. If the

erythrocytes are assumed all to have an equal lifetime of n days, the number of new erythrocytes arising, and the number of old erythrocytes removed from the circulation every day must both amount to 1/n of the total number of erythrocytes in the body.

Assuming that a blood transfusion to a normal individual does not affect his own production of blood corpuscles the plot for the destruction of the transfused corpuscles must take the form of a straight line. 1/n of the number of transfused corpuscles dies every day, thus the number of erythrocytes in the individual must diminish in a linear way until it reaches the number present before the transfusion, which will be after n days, the period corresponding to the lifetime of the corpuscles.

2. *The theory of destruction.* According to this theory the death of the erythrocytes is brought about by an active process. The amount of destruction which occurs depends on the number of corpuscles present but is completely independent of their age. If the daily destruction of corpuscles is equal to their daily production the number of corpuscles in the body may be maintained constant, regardless of whether it is the older or the younger corpuscles which are predominantly destroyed or whether corpuscles of all ages are attacked indiscriminately. For example 1/n of a given number of corpuscles is destroyed on the first day. On the next day 1/n of the remainder are destroyed and so on. In this case the destruction must be due to some organ destroying a certain proportion of the corpuscles in the blood. If this mechanism is present the amount of transfused blood in the body must diminish according to an exponential curve, and the corpuscles might reach an almost unlimited age.

Schiødt makes a reservation regarding the theory of longevity. He says that it is also possible that the process of active destruction predominantly affects the older cells ("it would appear uneconomical if cells were destroyed immediately after they had been created, and the consequence referred to, that some cells possibly should escape destruction, and last almost as long as the individual sheltering them, must also appear rather improbable"). If the variation in lifetime is very great between one cell and another, a proportion of them will die comparatively young, while others will become older so that the age-distribution of the corpuscles will approximate that expected according to the theory of destruction. However, he does not further discuss the form of destruction following from this possibility. He

further says: "In order to obtain a general view of the matter it has, however, been desirable to draw the contours sharply and rather push the matter to extremes."

Earlier different methods.

It is necessary to study the results which have been arrived at by earlier methods in order to obtain more precise information as to the mechanism by which transfused corpuscles are actually destroyed. Schiødt gives a good review of the work in this field.

The following are the chief methods which have been used previously:

1. Artificial raising of the number of corpuscles in the body and observation of the time taken before that number returns to normal.
2. Artificial diminution of the number of corpuscles in the body and observation of the time taken before that number returns to normal.
3. Introduction of blood of a different sort or type and microscopic or serological investigations to determine how long the corpuscles introduced remain in circulation.
4. Isolation or concentration in the plasma of the products of the destruction of corpuscles, urobilin and iron.
5. Calculations based on the reticulocyte count etc. in order to determine the age of the corpuscles.

The following table 3 (mostly derived from Schiødt's work) gives the values for the lifetime of the red corpuscles as found by various authors using different methods.

The different figures for the lifetime of the red corpuscle obtained by the different methods refer to the highest and lowest values found by the various authors.

The methods based on an *artificial raising or lowering* of the number of red corpuscles in the circulation are not sufficiently accurate to give definite information as to the method of destruction of the blood.

Ashby's (1919) *differential agglutination* method has been used by many workers, including recent ones.

The principle is that blood containing corpuscles which are not agglutinated by the recipient's serum, is transfused, for example group O blood into a group A recipient. A sample of blood is taken from the recipient after the transfusion

Table 3. Lifetime of red blood corpuscles according to different authors.

Method	Lifetime in days	Author
High altitudes	18—30	Haldane et al. (1913), Barcroft et al. (1923), Escobar & Baldwin (1934)
Transfusion	14—35	Müller & Jervell (1921), Isaacs (1937)
Regeneration after bleeding	33	Schiødt (1937)
Differential agglutination	15—120	Ashby (1919), Jervell (1924), Wiener (1934), Landsteiner et al. (1928)
Bilirubin	20—40	Bingold (1930)
Urobilin	54—200	Heilmeyer (1931), Lichtenstein & Landsberg (1931)
Iron	170	Cit. Schiødt (1938)

and to this is added a serum which agglutinates the corpuscles of the recipient but not those of the donor (in the given example a serum which agglutinates or haemolyses group A corpuscles). The corpuscles which have not been agglutinated (haemolysed) are counted and represent the number of corpuscles originating from the donor. However, the recipient himself has a certain proportion of corpuscles which will not be agglutinated and this must be determined before the tranfusion.

Very long lifetimes are often obtained using this and similar methods (see above). The method has been much criticised even by authors who have used it themselves. The technique is very difficult (Görl, 1926). This author observed that the number of non-agglutinable corpuscles belonging to the recipient varied very considerably. Rous (1923) points out that some of the material of the corpuscle stroma, which is itself antigenic, is not completely destroyed on the death of the corpuscle, but is partly used in the formation of new corpuscles. Thus some of the recipient's own corpuscles, formed after the transfusion, would give the same serological reactions as those of the donor and the value obtained for the lifetime of the corpuscles would be greater than the true value. Callender, Powell and Witts (1945) point out that the plot ought therefore to bo exponential in so far as the reincorporation of agglutinogen affects it. Isaacs (1924) also points out that the number of cells which are not agglutinated may be augmented by reticulocytes which have not yet acquired agglutinating properties arising in greater numbers owing to increased erythropoiesis after the transfusion. This would also lead to the plot for the destruction of the transfused corpuscles having an exponential form. Having regard to what has been said above, neither this method nor any of its many modifications can be considered to be suitable for the conduct of quantitative investigations. Furthermore the method is inherently liable to large errors.

Notwithstanding this, many authors still use the method for the purpose of determining the lifetime of the red corpuscles (Brown et al., 1944, Callender et al., 1945, Wiener, 1946).

Callender and his co-workers treat the problem of the destruction of erythrocytes thoroughly and mathematically using as a starting point experimental data arrived at by a modification of Ashby's method. The authors first describe the previous similar investigation of Brown and his co-workers which is said to agree generally with their own results. In many cases the destruction rate of the transfused blood was almost linear in the later part of its course. In order not to disturb normal erythropoiesis a venesection was first performed and the blood removed was replaced by the transfused blood. In the 4 experiments performed they obtained linear plots in two cases. In the third case the plot resembled an exponential curve at least during the first 20 days. The fourth plot was undoubtedly curved throughout its course. The first three cases were treated statistically. The result thus arrived at was that the plots for the destruction of the transfused corpuscles were linear in type within the margin of error of the method. Furthermore the authors draw the conclusion that all red corpuscles live for about an equal length of time, i. e. about 120 days. This appears to be a dubious result in view of the fact that the material consisted only of 4 cases, and that the course of the destruction was not the same in all of them; and also in view of the unreliability of the method. The other methods mentioned in table 3 have not been made to give any indication of the mechanism of destruction of the red corpuscles.

Trials with radioactive isotopes.

Hevesy and L. Hahn (1940) have tried to approach the problem of the life of the erythrocyte more closely by labelling corpuscles with *radioactive* P. There are however great obstacles to the use of this method in that almost all the compounds which are taken up by the blood corpuscles by means of physical or chemical combination leave them again.

One cannot count on the radioactive P remaining within the corpuscles for any length of time owing to the exchange of molecules between the corpuscles and the plasma and vice versa. Only molecules which are firmly united with the corpuscles and which have a longer lifetime than them would be suitable for such work. The compounds of radioactive P are thus unsuitable for use

as markers of erythrocytes, in any case when the time during which they must be observed is as long as that required for a study of the mechanism of the destruction of blood corpuscles.

The atoms of iron incorporated in the haemoglobin remain unchanged during the whole lifetime of the red blood cells. However, P. F. Hahn (1942) and his co-workers using *radioactive* Fe have found that the iron present in the haemoglobin of the destroyed corpuscles, is used preferentially in the formation of new corpuscles. This fact renders radioactive Fe unsuitable for use in the determination of the lifetime of the red blood corpuscles. Hevesy and Ottesen (1945) found that the P of desoxyribosenucleic acid is a suitable indicator for the determination of the length of life of nucleated red corpuscles. This substance is fixed within the corpuscles and does not pass into the plasma to an appreciable extent. The authors fed hens with phosphate containing radioactive P and were able to determine how long it was before all the corpuscles had reached equilibrium in respect of this substance, i. e. 28 days. Regeneration followed a linear course.

Shemin and Rittenberg (1946) in a recently published work have been able to study the concentration of *radioactive* N in the haemin of the red corpuscle after feeding human beings with glycine labelled with this isotope. These authors are of the opinion that the isotope is used in the construction of the corpuscle itself within the bone marrow and that it does not leave the corpuscle until its death when the isotope is not preferentially used again in the synthesis of new blood corpuscles. The authors arrive at the conclusion that the red blood corpuscles are not subject to indiscriminate destruction but have a definite life span which was found to be 127 days.

Trials with elliptocytes.

Another solution which seems more immediately applicable than the methods so far described is the use of corpuscles which are naturally labelled and which yet do not differ appreciably from normal corpuscles. *Elliptocytes* are well suited to fulfil these demands.

Huck and Bigalow (1923) transfused elliptocytic blood into a patient and found that the elliptocytes had disappeared after 2 months. Unfortunately they do not seem to have followed the elliptocyte concentration in the recipient in the meanwhile.

Vischer (1928) carried out 5 transfusions of elliptocytes and found that the lifetime of the corpuscles varied between 12 and 13 days. Vischer too did not follow the concentration of the elliptocytes continuously, so it is not possible to obtain any information from his results as to the mechanism of the destruction of the corpuscles. Vischer's estimate of the lifetime of the erythrocytes does not correspond with the results of other authors. It is impossible to find any definite cause for this discrepancy. His experiments were, however, not carried out by a very accurate method (see p. 25).

Kirkegaard and Larsen (1942) may be said to be the only workers who have hitherto tried, in a relatively painstaking way, to use elliptocytes in the study of the method of destruction of the blood corpuscles (see p. 25).

These workers carried out 3 transfusions of elliptocytic blood into persons with a normal blood picture and one transfusion of normal erythrocytes into an elliptocytic. In at least one of their plots, which was moreover the most regular of them, the points plotted lay very nearly on an exponential curve. In another case they obtained a plot of a similar shape, though less well-marked.

However, the authors quite rightly point out that their experiments cannot provide a basis for an opinion as to which of the theories is correct. According to the theory of longevity the lifetime of the red corpuscle is, on the average, 30—34 days. According to the theory of destruction the mean length of the lifetime is 11 14 days. There was a good correspondence between the curves obtained after the transfusion of elliptocytic blood into a normal person and after the transfusion of normal blood into an elliptocytic, which suggests that elliptocytes are destroyed in the same way as ordinary erythrocytes.

Sköld (1943) carried out a transfusion of normal blood into an elliptocytic. Using film preparations he demonstrated that the normal erythrocytes remained alive for at least 38 days in the elliptocytic blood, which corresponds quite well with the results of the last authors. However, the course of this plot also resembles an exponential curve.

Hedenstedt (1945), in a research on the transperitoneal resorption of blood corpuscles, showed that in 3 cases, elliptocytes introduced intraperitoneally were resorbed into the blood vessels and were still to be found in the circulating blood after 30 days. It is impossible to derive any information as to the mechanism of destruction of the red corpuscles from these figures either.

FOURTH CHAPTER.

Blood Volume.

There is a very voluminous literature dealing with the various methods which have been tried out in attempts to ascertain the blood-volume in animals and man. The present author does not intend to go into all the details in this field but only to give as brief as possible an account of such experiments and results in the earlier literature as may be of help in assessing the value of his own method. For an account of the classical literature the reader is referred to the exhaustive review of Erlanger (1921) and a monograph by Rowntree, Brown and Roth (1929).

Sources of errors in determination of the blood volume.

It has been difficult to determine the normal value of the blood volume, partly owing to imperfections in the methods used and partly to haemodynamic conditions which have been most closely studied by Fåhræus (1928, 1936).

This author made the following points. It has been known for a long time that under conditions of so-called "laminar flow" in a tube the infinitely thin layer of liquid which lies next to the wall of the tube remains motionless while the velocity of flow of the layers within this increase as the distance from the centre of the tube decreases and is greatest at the centre of the tube.

It has long been observed that when blood is flowing in the smaller vessels its chief components are not uniformly distributed over the cross-section of the vessels; the red corpuscles move in the centre of the tube (axial stream) while the plasma (and in some cases the white corpuscles) move in the peripheral stream. This observation means that the red cells have a greater velocity than the surrounding plasma. It is obviously impossible to determine the difference in velocity between the axial stream and the peripheral stream directly. Fåhræus has taken as his starting point the fact that the product of the relative concentration of corpuscles and the relative velocity in the vascular system must remain constant. He allowed blood to flow through glass capillary tubes of various calibres and determined the effects on the corpuscles and plasma. In this way he showed that the concentration of the corpuscles diminished as the diameter of the tube became less, this was specially marked for values below 0.5 mm. This dilution of the blood is one of the reasons for the fact observed by Fåhræus and T. Lindquist (1931), namely that the viscosity of blood diminishes with the diameter of the tube in which it is flowing, at first slowly but later rapidly. All vessels having a diameter of about 0.5 mm or less (including arteries, veins and such capillaries as are wide enough to allow of the formation of a peripheral stream) are thus grouped in

a special category within the vascular system with respect to the dilution of the blood in them and the decrease in its velocity. Fåhræus has called them *paracapillaries*. There is some support for the belief that the amount of blood present in these finer divisions of the arterial venous system comprises a large proportion of the amount of blood in the whole body. Fåhræus points out that this would explain the very considerable differences in the value obtained for the blood volume when it is estimated on the one hand by determining the total volume of the corpuscles and on the other by determining the volume of the plasma and then adding the correct proportion of plasma or corpuscles as the case may be, as obtained from the ratio corpuscles plasma in haematocritic estimations on venous blood.

The difference between the results obtained by these two methods may amount to 2 % of the body weight. In a person weighing 75 kg this corresponds to 1 $^1/_2$ litres. This difference is due to the fact that there is far more plasma and far fewer corpuscles in paracapillary blood than in venous blood. The difference in the values obtained for the blood volume would not, however, be so great unless a considerable proportion perhaps half, of the blood in the body was present in the paracapillary circulation.

In the same work Fåhræus mentions another circumstance which plays a part. There is reason to believe that the distribution of blood between the paracapillary vessels and the rest of the vascular system is not constant under different physiological circumstances. Assuming that the opening up of capillaries which were previously closed leads to the opening of more paracapillaries then more blood will come under the influence of the altered dilution mechanism, and there will be a relative increase in the number of corpuscles in the larger vessels (and also in the capillaries where the velocity of flow of the corpuscles and plasma is equal). An apparent increase of this kind in the total number of red blood corpuscles may be due to the effect of such a mechanism and need not be held to signify the emptying of a reservoir of blood corpuscles. On the other hand an emptying of such a reservoir may be completely concealed by a diminished activity of the dilution mechanism owing to more blood being discharged from the paracapillary vessels into the larger vessels. This mechanism, which was demonstrated by Fåhræus, seems to indicate that the distribution of corpuscles and plasma within the vascular system is a much more complicated phenomenon than was previously thought.

It may be said by way of summary, that *Fåhræus has pointed out that changes in the number of paracapillary vessels in action and in their diameter, may be a source of error in all methods for the determination of the blood volume which are not based on the summation of the results obtained by determination of the volume of the corpuscles with those obtained by determination of the plasma volume.*

P. F. Hahn et al. (1941), using red corpuscles labelled with radioactive Fe, showed that the number of red corpuscles was 25 % less than the number found by calculations based on determinations of the plasma volume and haematocritic measurements on venous blood.

P. F. Hahn et al. (1942) carried out similar experiments on dogs using radioactive Fe, and came to the conclusion that no appreciable amount of red corpuscles were immobilised in the spleen. The experiments lasted up to 24 hours.

Nylin (1945, 1946) using red corpuscles labelled with radioactive P came to the conclusion that there are no reservoirs which empty red corpuscles into the circulation after exercise, or that if they exist they are too small to have any appreciable significance. The experiment lasted for a good half hour after the injection.

However Barcroft's (1923, 1925) rediscovery of the function of the spleen as a blood reservoir as well as later investigations on dogs by the same author, show that blood corpuscles can remain locked up in the spleen in isolation from the rest of the blood.

The morphological basis for the function of the spleen as a blood reservoir has been demonstrated by Knisely (1936). He used transillumination of the spleen in experimental animals and showed that the red blood corpuscles remained locked up in the splenic sinuses during a period of 10 hours. The quantity of corpuscles which remain locked up in the spleen during these 10 hours is variable and is dependent on different physiological circumstances. Björkman (1947 — private communication) has also succeeded in verifying the fact that the sinuses of the spleen act as a blood reservoir.

It is usual to distinguish between the volume of *the circulating blood* and *the total blood* including the blood in the depots. The line of determination between circulating blood and depot blood in its wider meaning, varies from moment to moment.

Warburg (1943) states in a text book that the size and quantitative effects of the human blood depots are but imperfectly understood and that conclusions based on analogies with animal experiments, which are often themselves difficult to interpret, are of doubtful value. The true blood depot where the blood remains almost motionless in large sinuses, is in the spleen, and this is not thought to be so important in this respect, in man as in the dog. Depots have also been demonstrated in other organs such as the liver and the lungs. This should be very significant in connection with the physiological regulation of the circulation (Sjöstrand 1935).

From what has been said above it is obvious that *it is hard to define what is meant by the volume of the circulating blood* and, if this can be done, to estimate it. On the other hand it ought to be possible to obtain an idea of the *total blood volume* by the use of suitable methods.

When trying to determine the blood volume by dilution of a suitable indicator in the blood it is necessary that the indicator shall

not be eliminated before one can be sure that it has become evenly distributed throughout the whole volume of the blood. The indicator must thus remain substantially unchanged for a period of at least 10 hours after injection (see Knisely). The substances which have hitherto been used such as dyestuffs, carbon monoxide and radioactive P do not fulfil this requirement. The position is different in the case of radioactive Fe as this is firmly combined in the haemoglobin of the corpuscles and does not leave them until their death. However there is a certain difficulty in this case too, in that the Fe derived from dead corpuscles is taken up rapidly and selectively for the formation of new corpuscles (Cruz, Hahn et al. (1942), Ross and Chapin (1943)). Thus, at some time after the transfusion, the radioactivity of the corpuscles represents not only the surviving corpuscles from the transfusion, but also newly formed cells which become more and more numerous.

It follows from what has been said above that, in principle, the only satisfactory method of determining the blood volume is to determine the volumes of the plasma and corpuscles separately and add the values thus found. This procedure is independent of haematocrit readings, but assumes that a correct value is found for the plasma volume. Owing to the capillary dilution described above, which is of variable magnitude, haematocrit readings on venous blood or blood from the finger are too high. This affects the determination of the blood volume in the following way.

V = total blood volume. V_r = total volume of red corpuscles. V_p = volume of plasma.
R = Haematocrit reading.

1. Using primary determination of corpuscle volume $V = \frac{V_r}{R}$

2. Using primary determination of plasma volume $V = \frac{V_p}{1 - R}$

In the first case the estimated total blood volume will be too small if R is too large, because the extra amount of plasma in the paracapillary vessels is not taken into account. In the second case the estimated blood volume is too large by an amount corresponding to the volume of the corpuscles which were assumed to be present in the paracapillary blood but which were not in fact there. It would thus appear to be desirable to determine the plasma volume and the corpuscle volume

simultaneously and to add the values obtained together. Estimations of the plasma volume are, however, very unreliable.

The following are the chief methods which have been used. They may be divided into *direct* and *indirect* methods or into those in which the plasma volume is determined primarily and those in which the corpuscle volume is determined primarily.

Direct methods.

1. The oldest of these is Welcker's (1853) *exsanguination* method. In this method all the blood is washed out of the vessels and tissues by viviperfusion and the total amount of haemoglobin present is determined.

2. The *carbon monoxide* method is also among the direct methods aiming at a primary determination of the volume of the corpuscles. It was first introduced by Grenant and Quianquaud (1918) and has since been modified by many workers including Peters and van Slyke (1931). The values for the blood volume obtained in this way are sometimes too high and sometimes too low (van Slyke). The carbon monoxide method has the disadvantage that it is not possible to make corrections for the carbon monoxide which leaves the blood and is taken up by the muscle haemoglobin. (Asmussen 1942.)

Indirect methods.

1. Perhaps the most widely used and most widely discussed of the classical methods for the determination of the blood volume is that introduced by Keith, Rowntree and Heraghty (1915). They determined the plasma volume in the first place by introducing suitable *dyestuffs* and studying their dilution in the blood by colorimetry. However, much criticism has been directed against this method and all its modifications both in the earlier and in the more recent literature. King, Cole and Oppenheim (1943) as well as others, have investigated the properties of the dyestuff T-1824, which was recommended as suitable for use in blood volume estimations, and, in common with other authors, they reached the conclusion that the concentration of the dye in the blood falls quickly owing to the rapid diffusion of the dye into the tissues, endothelial system and lymph. The determination of the plasma volume is unreliable even when an attempt is made to make corrections for losses of dyestuff by extra-

pclation along the curves. P. F. Hahn et al. (1942) and Hevesy et al. (1944) have shown that the values obtained by this method are too high when compared with those obtained by the use of red corpuscles labelled with radioactive elements (P and Fe).

2. Determinations of blood volume have awakened increased interest in recent years owing to Hevesy's new methods of labelling red corpuscles with *radioactive isotopes.*

L. Hahn and Hevesy (1940) and Hevesy and Zehran (1942) devised a method for carrying out determinations of the blood volume using red corpuscles labelled with *radioactive* P. The method was worked out in detail in a research by Hevesy et al. who also discussed the sources of error in the method.

The experiments are carried out by adding phosphate containing radioactive P to 25 ml of whole blood, the mixture is then placed in a paraffin coated glass vessel and rotated in a thermostat at 34° C for 2 hours. The greater part of the mixture is then reinjected into the person on whom the experiment is being carried out. Hevesy and his co-workers discuss the errors which have to be taken into account when using this method, on the basis of certain experiments. Thus an error of 3 % is obtained owing to a small amount of active plasma adhering to the corpuscles, one of 5 % is obtained owing to the penetration of radioactive P from the plasma into the corpuscles of the recipient and one of 1.5 % owing to the loss of activity by the corpuscles if the test is carried out within 10 minutes of the injection. Hevesy et al. consider that these errors cancel one another to some extent and maintain that the total error is 0.5 % if the experiment lasts for 10 minutes, in the direction of making the corpuscle volume appear too small. The loss of activity was investigated in vitro.

Nylin (1945) has adapted this method for clinical use.

P. F. Hahn and his co-workers (1941, 1942) have devised a method using *radioactive* Fe.

When making determinations of total blood volume it is preferable to use an indicator which will certainly not alter within the blood vessels during a period of at least 10 hours, which is the shortest time that can be considered satisfactory for obtaining mixing in the splenic depot. None of the indicators used in the methods mentioned above retain their original values for so long a period. Thus dyestuffs rapidly vanish into the tissues, radioactive P leaves the corpuscles after at most one hour (Nylin). The use of radioactive iron appears to be the most reliable method, assuming that there is not time for an appreciable number of newly marked corpuscles to be formed during the time before complete mixing has occurred.

According to Warburg variations in the relative blood volume between one person and another amount to 25 %, and the different methods give completely dissimilar results. Hevesy et al. have carried out comparative experiments with the various methods, table 4.

Table 4. Different values of the blood volume obtained by different methods (Hevesy et al. 1944).

No.	Corpuscle volume determined by P_{32} method + plasma volume determined by the dye method	Blood volume determined by the dye method	Blood volume calculated from the corpuscle volume determined by the P_{32} method and the haematocrit figure	Blood volume calculated from the corpuscle volume determined by the CO method and the haematocrit figure
7	4930	5330	4300	5940
8	4830	5340	4240	5380
10	4420	4630	4140	7110
11	4910	5120	4690	6480
13	5110	5880	4540	6670
14	5170	5370	4760	5860
15	4810	5220	4330	6240
17	5130	5460	4430	5760
Mean value	4910	5300	4430	6180

The figures in the second column should theoretically represent the correct value for the blood volume. The corpuscle volume is determined by the use of P_{32} and the plasma volume by the use of a dye. The value is thus independent of haematocritic determinations, however it depends instead on the unreliable method for the determination of the plasma volume. In column 3 the blood volume is calculated from the plasma volume as obtained by the dyestuff method, which, as was to be expected, gives a value which is probably too high. When the corpuscle volume is determined as in column 4 an unduly low value should be obtained according to the argument put forward above. If the CO method were to give a correct volume for the corpuscles (column 5) the total blood volume calculated from haematocrit readings would be too low. According to this table the opposite is the case; this may be explained by the taking up of carbon monoxide by haemoglobin other than in the blood which thus overcompensates for the error.

Most of the workers with the methods referred to have supposed a very short *mixing time* of only a few minutes. Hevesy and his co-workers state the mixing of the active and inactive corpuscles may possibly not have occurred within 3 minutes of the injection. Nylin maintains that if blood corpuscles labelled with radioactive P are injected into a normal experimental human subject it is found that they quickly become mixed with the circulating blood. In certain cases this occurs before or at the end of the first minute after the injection, and usually before the fifth minute. "The injected corpuscles then retain their activity constant for a long while after the injection, even for as long as 1 hour." P. F. Hahn and his co-workers, working with dogs, find that mixing of the radioactive corpuscles throughout the whole vascular system occurs during the course of 2 minutes. On the other hand Gibson and Evans (1937) using a dye, found that mixing was not complete until much later.

FIFTH CHAPTER.

Transperitoneal Resorption with Particular Reference to Corpuscular Elements.

As this work is primarily concerned with the resorption of *corpuscle* elements from the peritoneal cavity, the author wishes, in reviewing the literature, to pay special attention to studies dealing with, or having a bearing on this part of the problem of transperitoneal resorption. From a physiological point of view it is of chief importance to ascertain how effectively and how rapidly the resorption takes place. The problems of the path of resorption and the mechanism of resorption are so intimately bound up with one another that no attempt has been made, in what follows, to discuss them separately.

Lymphatic drainage.

von Recklinghausen (1862) appears to have been the first to study in any detail the phenomena of resorption from the peritoneal cavity. Recklinghausen who introduced into the literature the terms *stigmata and stomata*, supposed connections between the peritoneal cavity and the lymphatic system. He considered that he could

demonstrate, by silver nitrate staining, the existence of openings between the peritoneal epithelial cells leading into a subserous lymphatic plexus. Recklinghausen made histological investigations of excised portions of the diaphragms of animals. He poured ink, milk, cinnabar etc. on to these pieces, and observed a resorption of the material on stretching the tissue. During the resorption he could, with the dissecting microscope, observe swirling of the suspensions in the vicinity of stomata, together with a streaming of the absorbed material along the subserous lymph vessels. The majority of Recklinghausen's contemporaries accepted his views.

Other workers of the same period, however, tried to prove that Recklinghausen's stomata were artefacts, due to precipitation of silver chloride or silver albuminate on the peritoneum, e. g. Oedmanson (1863). Kolossow (1893) using an osmic acid staining method, claimed to demonstrate intercellular channels in the peritoneal mesothelium, while, in his opinion, direct connections with the subendothelial lymph vessels were absent.

In the controversy which continued on the subject of peritoneal resorption, MacCallum (1903) stated that he could find no evidence for the existence of stomata, and regarded the peritoneal cavity as being completely closed. According to MacCallum carbon particles injected into the peritoneum are taken up by phagocytes, which wander through the serosa and reach the lymph vessels in the dome of the diaphragm. Other free particles were supposed to be resorbed in the same way. MacCallum, however, considers that, to explain satisfactorily the passage of these corpuscles through the peritoneal epithelium and lymphatic endothelium to the subserous lymph spaces, it is necessary to suppose that the connections between the cells of the endothelium are so frail that the powerful pumping action of the diaphragm is sufficient to force the material between the cells. The subserous lymphatic net-work which is here involved is referred to by MacCallum as *lacunae*. A particle in the peritoneal cavity, in passing from there to the lymphatic network, must first traverse the three-layered membrane separating the two spaces. First the peritoneal epithelium and the basal membrane must be penetrated, and then the lymphatic endothelium, the "lining of the lacuna". On entering the lacuna, the particle is passed rapidly to the larger lymph-vessels, which may readily be observed through the pleura, and which receive lymph both from the pleura

and the peritoneum. The lymph-glands above the diaphragm are observed to contain particles after quite a short time.

However, MacCallum took the view that there was no difference in principle between his own and Recklinghausen's picture of the process, at any rate from a practical point of view. According to the one theory, direct and open connections were pictured between the abdominal cavity and the lymph-vessels, having the form of channels filled with liquid; according to the other, the same functional relationship exists, on account of loose connections between the cells. MacCallum further found that if a dead dog was subjected to the motions of artificial respiration, intraperitoneal particles could be driven into lymph vessels, above the diaphragm.

v. Recklinghausen's observations have not gained general credence. Nevertheless it should be pointed out that the existence of peritoneal stomata has never been disproved. The controversy as to the existence of these stomata was very violent at the close of the 19th century, as is evident from the voluminous literature on the subject, which has been carefully listed by Cunningham (1926).

Higgins and Graham (1929) showed that the lymph vessels of the dog are larger and more numerous on the right half of the diaphragm; this was beautifully demonstrated by intraperitoneal injection of graphite particles. Higgins employed a window in the thoracic cage, through which he could clearly observe the injected lymph vessels on the dome of the diaphragm. According to this author, the particles are chiefly resorbed by five different routes. The greatest part of the absorbed material goes by the so-called "sternal" path. This comprises lymph vessels which leave the diaphragm and run between the intercostal muscles parallel with the sternum. These vessels drain into a series of lymph glands between the first and third rib, and then into the right lymphatic duct and the thoracic duct. About 80 % of the material is resorbed along these paths. The second route is the "pulmonary" route, and consists of small vessels which pass through lymph glands in the hilus of the lung and anterior mediastinum. The third route appears to have no great significance for the transport of particles, and to consist of small lymph vessels debouching into the thoracic duct. The remaining routes are relatively of little significance, and pass through the diaphragm, emptying their lymph in the region of the kidneys and pancreas. After injection of graphite the particles are seen in the

sternal lymph vessels after 12 minutes, and only a little later reach the thoracic duct. The pulmonary lymph vessels are also more efficient than the thoracic duct as regards drainage from the abdominal cavity. Communication exists between the lymph vessels of the pleura and those of the peritoneum.

Higgins and his collaborators (1930) showed that if the phrenic nerve in the dog is cut unilaterally, there is a delay in the progress of resorption on the paralysed side. Only much later, when muscular atrophy has set in, does the velocity of resorption again increase.

Florey (1927) endeavoured to study the pressure inside the peritoneal cavity by inserting, between the liver and the diaphragm, a rubber catheter fitted with a rubber balloon of 1000 ml capacity. He observed, during the respiratory movements, the development of a pressure difference amounting to 3—5 cm water. Immediately under the diaphragm the pressure was always below atmospheric pressure, owing to the influence of the thoracic cavity. Florey also pointed out that the abdominal muscles of these experimental animals were completely relaxed during the measurements on account of the anaesthesia, and that slight pressure with the hand on the abdomen increased the pressure by several cm water. Florey considered that the intra-abdominal pressure is of decisive importance for the resorption, and that most of the resorbed material is forced between the endothelial cells of the diaphragmatic peritoneum.

Watkins and Fulton (1938) measured the flow from a cannula in the thoracic duct after injecting 400—1000 ml of such fluids as water, physiological saline, 6 % gum acacia, horse serum and blood into the peritoneal cavity. Only in one instance, the experiment with gum acacia, was there any marked increase in the flow of lymph in the thoracic duct.

Particle resorption.

On the question of the resorption of particles from the peritoneum to the blood, a clear-cut picture was first obtained by Allen (1936) and Allen and Vogt (1937). Allen showed definitely and clearly by histological methods with mice that stomata in the peritoneum are normal and physiological openings connecting with the lymphatic lacunae. It is through these openings in the serosa that fluid from the peritoneal cavity comes into direct contact with the lymphatic endothelium. The openings which were originally observed by Reck-

linghausen represent the peritoneal mouths of short channels which pass through the peritoneal epithelium and the basal membrane to the lymphatic endothelium. It is, according to Allen, very significant that these stomata only occur at the sites of the subserous lymph spaces or lacunae, and not on other parts of the peritoneum. The histological relationship between a lacuna and the serous membrane is shown in a figure. In the picture the roof of the lacuna appears to be raised above the surface of the diaphragm; this is due to the fixation process, and in the un-fixed diaphragm the roof is actually depressed in relation to the diaphragm, so that the volume of the lacuna is diminished. Allen also gives a beautiful photograph of nucleated blood corpuscles from a frog passing through the roof of the lacuna, see fig. 22. In a preparation of this kind Allen could count up to 600 such blood corpuscles in the act of being absorbed. The lymphatic lacunae and the channels leading out from them also contained many nucleated blood corpuscles. The diameter of the stomata was estimated to be in most cases 2 --6 μ, but the author states that a number of the stomata were markedly larger. The blood corpuscles of the frog are considerably larger than those of the mouse.

A question devoid of physiological significance, but nevertheless of great histological interest is to what extent these stomata are real anatomical communications between the peritoneal cavity and the lacunae, or whether they are purely functional in character. It is evident that the stomata are proper openings at least at that instant when corpuscular resorption takes place.

Allen, and Allen and Vogt were able to show that the particles are sucked through the stretched peritoneum during relaxation of the diaphragm. If a few drops of Indian ink are poured on the peritoneal surface of the diaphragm, and the diaphragm is then stretched, one can immediately observe how the Indian ink has passed through the roof of the lacunae, and gained admission to the lacunae themselves. With the microscope one can observe that the roofs of the lacunae are lifted, during the stretching process, from their decumbent position, in such a way that the volume inside the lacuna increases. This results in a negative pressure inside the lacuna, which leads to a sucking in of suspensions and solutions through the stomata. According to Allen's investigations, the only barrier between the abdominal cavity and the lacunae consists of intercellular substance

in those regions which correspond to stomata. It appears that there occurs a dehiscence in the lymphatic intercellular substance at the locality of these stomata when the membrane is stretched and definite openings result, at least temporarily, through which the sucking-in process can take place.

The filling of the terminal subserous lymph-vessels is thus connected with the dilatation and stretching of those tissues in which the lymph-spaces are situated; the continued flow to the lymph plexus higher up is the result of compression of the lacunae, owing to pressure from the surrounding tissue. The absorption of intraperitoneal lymph should thus be distinguished from the lymph-flow itself. Retrograde filling of the lacunae is prevented by valves in the lymph-vessels. Practically the lacunae increase in volume when the diaphragm is relaxed, that is on expiration. A pressure difference is then established on opposite sides of the sero-endothelial membrane, which separates the abdominal cavity from the lymph-spaces, and the particles are thus sucked into the lacunae. The sero-endothelial membrane is thus, from the physiological standpoint, to be regarded as a sieve during the process of corpuscle resorption; penetration of the membrane and the filling of the lymphatic cavity is an almost instantaneous process.

The mechanism of compression of the lymph-spaces during inspiration, when the diaphragm contracts is rather different in different species. In those animals, such as the dog, man, and the mouse, where the lymph-spaces are confined to the muscular part of the diaphragm, it is necessary that the diaphragm should be compressed against the liver. In the rabbit the pressure from the viscera is not necessary, because "the lymph-vessels are so arranged in the tendinous central part of the diaphragm that simple contraction of the diaphragm compresses the plexus".

Allen and Vogt also considered that they had shown experimentally that the principles governing resorption through the diaphragm are also applicable to other serous cavities — e.g. pleura, pericardium. Miller (1937) considered that the normal pleura does not possess any stomata. As Drinker and Yoeffey point out one must not forget that during the movements of breathing the external epithelium of the lung and the pleura must stretch and relax, and thus afford the same conditions as the diaphragm for the coming into being at least of "functional stomata". These last authors maintain that the drainage of lymph from the pleura is a very slow process.

Efskind (1940) discussed in a very thorough way the problem of the resorption of particles from the peritoneum. In his long monograph he describes experiments with rabbits, designed to elucidate the course of resorption from the peritoneum under all conceivable circumstances in physiological and pathological conditions. The technique consists both of direct injections into the lymph-paths followed by histological investigation and of studies of the resorption of various substances under differing conditions, e.g. taking X-ray photos after injection of thorotrast in peritoneum. His conclusions may be summarised as follows. He found no evidence for stigmata or stomata, but came to regard the peritoneal cavity as closed. Regarding the course of the lymph vessels concerned with transport from the diaphragm to the blood, Efskind arrived, on the whole, at the same result as Higgins and his co-workers. He emphasizes, quoting experimental support, that the thoracic duct has only a slight significance in peritoneal resorption. He regards the diaphragm as an organ of great importance for resorption. The elimination of particles from the peritoneum takes place exclusively along lymph-paths for which the diaphragm plays a paramount role. The sucking-in through the diaphragm occurs chiefly through the right dome, for which the proximity of the liver is regarded as significant. Other parts of the peritoneum play an insignificant role in the resorption of particles from the peritoneal cavity. Efskind does not enter on a discussion of the mechanism of the resorption process itself, as studied by Allen and Vogt who, indeed, do not figure in his reference list. However he demonstrates that the peristaltic movements of the intestine, as well as the movements of the diaphragm, determine to some extent the distribution of particles within the peritoneal cavity. Paralysis of the intestine has a slowing effect on the resorption process. This can be observed, for example, after laparotomies where postoperative intestinal paralysis results. If particles are injected intraperitoneally, the resorption is at first rapid but after 24 hours has a tendency to fall off. At the same time one finds compacted masses of particles in the efferent lymph channels and their local lymph glands. There thus arises to some extent a blockage of the efferent lymph paths. This, however, is not absolute.[1]

[1] Note in the proof-sheet: When the present work was in the press, Holm Nielsen (1947) published a paper treating of resorption of exudates from the peritoneum.

Resorption of Red Blood Corpuscles from the Peritoneal Cavity.

The resorption of blood from the peritoneal cavity has always been a subject that has attracted attention. Many authors have described the transfusion of blood into the abdominal cavity in animals, and have established that the blood disappears rapidly. The question has also arisen whether the corpuscle elements — particularly the erythrocytes — are resorbed intact into the circulation in a functional condition or whether, either before or during the resorption, the red blood corpuscles undergo destruction and are perhaps resorbed in a haemolysed state. This question must have considerable significance not only for experimental biologists, but also for surgeons, since when they encounter extensive abdominal bleeding they are concerned whether the blood should be completely removed or left in the hope that it will be spontaneously resorbed. Of course the decision taken on this question depends also on such other factors as the risk of infection, the formation of adhesions, etc. A clarification of the problem of resorption of corpuscles from the peritoneum should also be of interest to pediatricians, since the possibility exists, in cases where suitable veins for intravenous transfusion are absent, of administering the blood intraperitoneally. Such transfusions have been given therapeutically for more than 50 years now, and many authors are impressed with the effectiveness of this method. The method is also described in some modern text-books. Although it is by no means generally employed.

Experiments with animals demonstrating blood resorption from the peritoneal cavity.

Recklinghausen (1862) injected de-fibrinated blood intraperitoneally, and found that a resorption took place. Ponfick (1875) furthermore established that an increase in the number of red corpuscles in the blood occurred 1—2 days after the injection. Rapid and complete resorption of blood was also demonstrated by Poncet (1879). Obalinski (1880) found that the increase in blood corpuscles in the circulation was proportional to the quantity injected.

Hayem (1884), working both on rabbits and dogs, made very thorough studies of this question, and concluded that there is a rapid

and complete resorption of blood, whether de-fibrinated or not, from the abdominal cavity. He was unable to detect any pathological changes in those blood corpuscles that had not already been resorbed. In order to settle the important problem whether the cells are resorbed living and intact, he hit on the idea of injecting dog's blood intraperitoneally into rabbits, with the purpose of recognising the much larger dog corpuscles in the rabbit's blood. This he succeeded in doing. He concluded that an intraperitoneal transfusion could be regarded as identical with a slowly administered intravenous transfusion.

Hunter (1886) showed, on the rabbit, that an amount corresponding to 32—92 % of the animal's own blood could be injected intraperitoneally without bad effects. After the injection, he observed a slight increase in the number of blood corpuscles on the following day, a subsequent decrease on the next day, and then a more permanent increase with a maximum on the 5th—6th day, and lasting for about 26 days. He obtained the same result with de-fibrinated blood.

Lesage (1900) found in animals that blood transferred by auto-transfusion to the abdominal cavity was completely resorbed within 48 hours; after this time the abdominal cavity was completely normal. He carried out an elegant experiment in which, immediately after an auto-transfusion into the abdominal cavity, he made a fistula from the thoracic duct. The lymph, which at first appeared normal, became pink after 45 min. and then gradually more and more the colour of blood. Microscopic investigation revealed masses of normal red blood corpuscles in the lymph.

According to Lennander (1902) resorption of different particles and red blood corpuscles takes place from the peritoneum to the circulating blood through stomata in diaphragm along lymphatics.

Zimmerman (1921) found that the blood corpuscles of birds, when injected intraperitoneally in the rabbit or guinea-pig could be found after a short time in the circulation of these animals.

Siperstein and Sansby (1923) showed that a rabbit could resorb an amount corresponding to about 1/5 of its own blood volume in 4 hours They further found that the intraperitoneal injection produced an immediate increase in the blood count and percentage of haemoglobin. This was temporary during the period of the resorption, but at a later stage there occurred a definite and permanent increase in the

cell count and the percentage of haemoglobin. Using undiluted blood in transfusion, resorption did not result with certainty: coagula probably formed in the abdomen. For this reason the experiments were done with citrated blood.

P. F. Hahn and co-workers (1944) demonstrated resorption of red blood corpuscles from the abdominal cavity of the dog by using erythrocytes "labelled" with radio-active iron. The experiments were too few in number to give any certain quantitative data on the resorption. A substantial fraction of the blood corpuscles was resorbed within the first 24 hours. If, employing their largest series (8 anaemic dogs), one calculates the average amount of blood resorbed after different times, a maximum is encountered in the resorption curve about 6 –7 days after the injection. The other series are too small and imperfect for making such a calculation (5 normal animals and 3 with hypoproteinaemia). There is, however, no evidence that the process differs here. If means are also calculated for the haematocrite values, these show no definite rise in the 8 anaemic dogs during the short observation period of five days. Judging by the published figures, the peritoneal resorption varied very much in the different dogs at different times. As pointed out p. 32, experiments of longer duration cannot be done with radio-active blood corpuscles, so that the viability of the blood corpuscles after resorption cannot be determined in this way. These authors were also able to show histologically that intact red cells rapidly passed from the peritoneal cavity to the lymph-spaces in the diaphragm and other parts of the peritoneum. The red blood corpuscles were transported along the lymph-vessels and through the lymph-glands with little or no phagocytosis occurring. The lymph vessels in the anterior mediastinum contained blood corpuscles, but the thoracic duct in mid-thorax contained none.

Clinical experiences which demonstrate resorption of blood from the peritoneal cavity.

As early as 1875 Ponfick injected de-fibrinated blood into 3 patients with good outcome. Golgi and Raggi (1880) likewise transfused de-fibrinated blood to a 30-year old patient intraperitoneally. After 18 hours an increase in the patients haemoglobin was observed. After 14 hours more the haemoglobin had risen further.

Siperstein and Sansby (1923) gave a trial intraperitoneal transfusion to a child, and observed an increase both of haemoglobin and of red blood corpuscles.

As regards the danger of blood being left in the abdomen, Siperstein and Sansby's review of the literature shows that very divided opinions have been held on this matter. Some of the authors point to the danger of infection and the formation of adhesions, while others consider it of value to have blood left in the abdomen after an intra-abdominal haemorrhage. Most, however, take an intermediate position, removing only large coagula, and leaving liquid blood in situ. It does not seem possible to adduce decisive arguments in favour of either point of view. The procedure adopted seems generally to be conditioned by the personal experiences of the various clinicians. Some surgeons have, with success, retransfused intravenously blood found on operation in the abdominal cavity in trauma or extrauterine pregnancy. (Lichtenstein, 1918; Ranft, 1917; Kreuter, 1916, and other authors.) "It is a well-known fact that women, who have lost large quantities of blood owing to a ruptured tubal pregnancy, recover at a surprising rate, whereas patients who have suffered severe external bleeding do not recover so rapidly from their anaemia" (Siperstein and Sansby).

Opitz and Metis (1924) carried out intraperitoneal transfusions on human beings and, on the basis of cell counts and haemoglobin estimations on the blood, thought that they had established that both plasma and blood corpuscles are completely resorbed. Serum was resorbed faster than the blood corpuscles. The resorption had usually begun after a few hours, and was complete after 16 hours. In general, however, the resorption required 3—4 days. The liquid blood was naturally resorbed faster than the coagula. With defibrinated blood, autopsy revealed no damage whatsoever to the peritoneum or viscera. The time of the observation varied from 7 hours to 30 days after the injection.

As mentioned above, many attempts have been made to demonstrate resorption of blood corpuscles from the abdominal cavity by means of red cell counts and haemoglobin determinations on the circulating blood. Babies form the most usual clinical material. All the experiments are open to criticism, namely that it is impossible to demonstrate that the increase is due to a simple transfer of corpuscles from the abdominal cavity. It is theoretically conceivable that both

the haemoglobin and the red cell figures can rise as a result of increased production by the bone marrow acting under stimulation from the injected blood. Furthermore, particularly with babies, on account of their variable state of hydration, etc., red cell counts are very unreliable. It is therefore impossible to regard these clinical experiments as having provided any conclusive evidence for the resorption of red blood corpuscles from the peritoneal cavity.

Yet it appears from the relevant literature, highly probable, that in man resorption occurs from the abdominal cavity into the circulation. This view has been particularly strengthened by the study of analogous conditions in experimental animals. However, conclusive proof has hitherto been lacking, particularly in respect to the corpuscular elements. Of course, convincing proof can only be obtained using an analogous procedure to the animal experiments with corpuscles from other species or labelled in some other way. This implies that one must recognise in the circulation blood corpuscles that have been injected intraperitoneally.

This idea seems first to have been put into practice by Clausen (1940), who using a modification of Ashby's (1919) method, considered that he had shown that at least some of the red blood corpuscles administered intraperitoneally to children pass from the peritoneal cavity into the circulation. The method is, in outline, the following.

The child's blood group in the ABO and MN systems is determined. A donor of the same ABO group but of the opposite MN group is selected. (If the child belongs to group M, the donor is taken from group N, and vice versa.) Using agglutination tests with anti-M and anti-N sera, it is possible to distinguish between the corpuscles of the child and those of the donor.

However, Clausen was unable, using this method, to make a definite estimate of the quantity of the donor's corpuscles which had passed through the peritoneum into the circulation. The procedure has been criticised in chapter 3, p. 30. However, it is probably legitimate to consider that, despite the inaccuracy of the method, Clausen obtained the first direct proof that a resorption of blood corpuscles in man through the peritoneum into the circulation really occurs. It was impossible in this way to obtain any idea of the behaviour of the corpuscles after resorption.

Winzeler (1945), independently of the previous author, made similar experiments employing a different modification of Ashby's method.

The principle was, in a mixture of O and A bloods, to effect isolation of the O corpuscles by haemolysing the A corpuscles by means of a haemolytic serum acting specifically on them. In other respects the procedure was the same as Clausen's. This method is very laborious and complicated, and the quantitative aspects of the problem could not be handled.

Winzeler did, however, establish that resorption occurred.[1]

It seems thus that the problem of the resorption of blood corpuscles from the human abdominal cavity has not been unequivocally solved, at least in its quantitative aspects. The important questions concerning the quantities resorbed, the speed of resorption, the functional capacity of the corpuscles after their stay in the peritoneal cavity, etc., are still unsettled. This seems entirely due to the fact that methods suitable for the purpose have not previously been available.

In a short communication Hedenstedt (1945) showed that this problem could be handled quantitatively, using elliptocytes as labelled corpuscles. In 3 cases it was shown that elliptocytes injected into the human peritoneal cavity were for the most part resorbed into the circulation in the course of 6 days.

Summary of Previous Investigations on the Resorption of Corpuscular Elements from the Peritoneal Cavity.

I. *Animal Experiments.*

1. Suspensions of particles are resorbed rapidly along the lymph channels from the peritoneum.
2. The resorption takes place almost exclusively through the diaphragmatic peritoneum into the lymphatic plexus (lacunae).
3. Differing views are held concerning the paths of resorption. The investigations of Allen seem, however, to show that stomata, at least of a functional sort, exist in the serosa that covers the diaphragm. The question whether the stomata are "anatomical" or "functional" in nature is moreover chiefly of morphological interest.

[1] Winzeler also considered that with the same method he could show a resorption of intact red blood corpuscles from the rectum into the blood stream. This, however, seems very improbable and goes to prove that the method is not reliable. In experiments under the same conditions as Winzeler, but using elliptocytes, the present author found that such a resorption does not take place.

4. As regards the mechanism for absorption of the material through the stomata divergent opinions also prevail. The study which seems to provide the most reliable information on this point is that of Allen and Vogt. The absorption appears to be effected by a suction mechanism inside the terminal lymphatic plexus (lacunae) of the diaphragm, which is brought into action by the relaxation of the diaphragm on expiration. The lymph-flow seems to be occasioned by compression of the terminal lymphatic plexus against the liver during inspiration in the higher mammals. Retrograde flow is prevented by valves inside the lymph-vessels.
5. Some investigations indicate that the peristaltic movements of the intestine as well as the movements of the diaphragm are significant factors in distributing the injected material within the abdominal cavity. In addition the negative pressure prevailing in the upper part of the abdominal cavity probably plays a decisive role.
6. The absorbed material is passed on chiefly via the retrosternal lymph-vessels to the subclavian veins of the neck. The lymph-vessels traverse lymph-glands.

II. *Human Experiments.*

1. Some human experiments in which blood was injected into the abdominal cavity, indicate that blood corpuscles can be resorbed into the blood from the abdominal cavity without phagocytosis occurring.
2. No reliable experimental data are available yet regarding the transperitoneal resorption of blood corpuscles. This appears to be due to the fact that workers in this field have not yet had any suitable experimental method at their disposal. It is thus not yet known whether the erythrocytes introduced into the abdomen undergo any changes, e. g. haemolysis, occasioned before or by the resorption. Nor is it known how much of the blood has been resorbed after different times. Finally there is no evidence of the blood corpuscles retaining their functional capacity unimpaired after resorption, or on other relevant questions.
3. The author considers on preliminary experiments that it is possible to obtain elucidation of these unsolved problems with the aid of transfusions of elliptocytes.

Part II. The Author's Experimental Investigations.

SIXTH CHAPTER.

Material.

The material used in the present research consisted partly of elliptocytics and partly of other experimental subjects.

Elliptocytics. These are 12 in number and comprise 5 women and 5 men from 3 families. 2 cases are isolated ones. The ages vary between 15 and 70 years.

They were all, generally speaking, healthy. A detailed account of the properties of their blood will be given in a later chapter. The work of finding elliptocytics has been greatly facilitated by the hereditary nature of the anomaly. When a case was detected by chance a search among the relatives usually revealed new cases. As there was no dearth of willing blood donors for these experiments the author did not have to go to great trouble to find more elliptocytics, though this might be necessary if experiments using the elliptocyte method were to continue. The anomaly probably passes unnoticed if people are not on the look-out for it. See p. 13 for information as to its frequency. The diagnosis is very easy to make for anyone who is at all used to it, owing to the characteristic shape of the blood corpuscles. The finding of signs of its hereditary nature further confirms the diagnosis.

Experimental subjects. These include 51 persons, 1 man, 1 woman and 49 children, the youngest 8 days and the oldest 13 years old. (Some of the persons were used twice as experimental subjects with an interval between. This is marked in the table with a small letter before the number of the experiment. Those having the same letter were carried out on the same subject.) As is seen from table 5 most of them were suffering from various illnesses. Some of the children were anaemic according to the limiting values for haemoglobin given in chapt. 8, the anaemias were of the hypochromic type. Concerning the diagnosis, it may further be mentioned that 11 of the

Table 5. The experimental subjects.

Case	Sex	Age	Body weight, kg	Hæmoglobin, %	Erythrocytes, mm^3	Hematocrit, %	Elliptocytes, %	Transfusion	Diagnos
I_2	♀	1 month	1.69	90	4.810	—	0.48	i. p.	Debilitas vitae congenita
I_3	♀	23 days	1.74	90	4.550	—	0.76	i. p.	Debilitas vitae congenita
I_4	♀	14 »	2.01	95	4.555	—	0.4	i. p.	Debilitas vitae congenita
I_5	♂	13 »	2.24	105	5.025	—	0.75	i. p.	Debilitas vitae congenita
I_6	♀	1 year 7 months	8.26	93.5	5.195	—	0.97	i. p.	Coeliakia
I_7	♂	3 years	10.47	77.5	4.835	—	0.11	i. p.	Coeliakia
I_8	♂	3 » 3 »	14.7	74.5	4.295	—	0.16	i. p.	Nephritis acuta
II_1	♀	3 months	4.04	84.5	4.370	—	0.16	i. p.	Hydrocephalus internus
II_2	♂	40 days	2.54	112	4.705	—	0.39	i. p.	Dystrophia
II_4	♂	3 years 2 months	17.8	75	4.870		0.74	i. p.	Exzema infantum
III_1	♀	8 days	1.04	137	4.750	—	0.44	i. p.	Debilitas vitae congenita
III_2	♂	45 »	2.41	74	2.450	—	0.30	i. p.	Debilitas vitae congenita
III_3	♀	25 »	2.94	102	4.615	—	0.27	i. p.	Malformatio nasi
III_4	♀	4 »	1.45	112	4.770	—	0.50	i. p.	Debilitas vitae congenita
III_5	♂	4 months	4.17	61	3.480	—	0.15	i. p.	Pneumonia acuta
III_6	♂	$5^1/_2$ »	6.3	55	3.980	—	0	i. p.	Empyema pleurae + Anaemia secundaria
III_7	♂	9 years	28.6	78	3.920	—	0.41	i. p.	Vitium organicum cordis
III_8	♂	1 year 4 months	6.5	71	4.350	—	0.53	i. p.	Chondrodystropha foetalis
III_9	♂	5 » 9 »	22.5	82	5.490	—	0.11	i. p.	Tuberculosis pulmonis primaria
III_{10}	♂	8 days	1.14	124	3.855	—	0.18	i. p.	Debilitas vitae congenita
IV_3	♂	7 months	7.14	72	4.100	—	0.16	i. p.	Empyema pleurae
IV_4	♀	18 days	2.6	105	5.160	—	0.37	i. p.	Cheilognathopalatoschisis
IV_6	♂	18 »	3.1	96	4.615	—	0.12	i. p.	Vitium conformationis
IV_7	♀	13 years	23.9	69	4.560	—	2.35	i. p.	Vitium congenitum cordis
V_4	♀	2 »	13.1	62	4.865	—	1.29	i. p.	Pleuritis exsudativa tuberculosa

V_6	♀	52 days	2.61	91	4.915	—	0.44	i. p.	Debilitas vitae congenita
IX_8	♀♀	3 years 7 months	10.35	56	4.978		0.17	i. p.	Enteritis + Anaemia secundaria
X_2	♀♀	2 » 3 »	11.0	62	—		0.32	i. p.	Nephrosis
X_5	♀♀	9 months	6.35	80				i. p.	Hydrocephalus internus
IX_6	♀♂	1 day	2.9	—	—	—	—	i. p.	Acrania
IX_7	♂	1/2 day	2.54	—	4.915		0.27	i. p.	Acrania
IV_2	♀	32 years	78	89	4.910	35.1	1.04	i. v.	Nephropathia gravidarum
IV_8	♂♀	6 »	21.8	72	4.530	40.2	0.08	i. v.	Pertussis
V_2	♀♀	9 » 6 months	23.7	72	4.540	38.6	0.03	i. v.	Empyema pleurae
V_3	♀♂	12 »	31.1	76	4.540	38.6	0.19	i. v.	Polyarthritis acuta
a VI_3	♂	9 months	4.6	48	4.895	44.3	1.20	i. v.	Enteritis cum dystrophia + Anaemia secundaria
VI_4	♀♀	2 years 2 months	7.2	66	4.155	37.6	0	i. v.	Coeliakia
VII_1	♀♀	8 months	5.09	84	—	39.4	0.05	i. v.	Paedatrophia
b VII_2	♀	6 »	4.18	75		28.8	0.04	i. v.	Debilitas vitae congenitas
a VII_3	♂	1 year 3 months	9.53	64		26.7	0.34	i. v.	Enteritis cum dystrophia + anæmia secundaria
e VII_4	♂	1 » 3 »	8.6	64		31.5	0.54	i. v.	Enteritis chronica
c VII_5	♀♂	1 » 9 »	9.13	71		31.3	0.37	i. v.	Nephrosis
$VIII_6$	♂♀	2 » 5 »	8.29	69	—	40.0	0.14	i. v.	Dystrophia
d IX_2	♀♂	3 » 8 »	10.1	63		30.4	0.23	i. v.	Coeliakia
IX_3	♂♀	3 » 8 »	18.9	75	—	31.9	0.09	i. v.	Entero-colitis subacuta
c IX_4	♀♂	2 »	10.32	53	—	21.6	0.43	i. v.	Nephrosis
e X_1	♂♀	1 » 10 »	11.17	82	—	41.0	0.22	i. v.	Enteritis chronica
b X_3	♀♀	1 » 1 »	8.95	57	—	26.7	0.47	i. v.	Debilitas vitae congenita
d X_4	♀♀	4 »	12.19	71	—	35.5	0.06	i. v.	Coeliakia
XI_2	♀♀	12 »	33.6	75	—	31.0	0.25	i. v.	Nephritis acuta
XII_1	♂	53 »	—	86	—	36.7	0.26	i. v.	Fractura complicata

children were being treated under the diagnosis of congenital debility, 7 of the cases had congenital deformities and the rest were under treatment for nutritional disorders and infectious diseases.

The table also gives their elliptocyte counts and whether the transfusions were given intravenously (i. v.) or intraperitoneally (i. p.). The cases are numbered in the same way as in the experiments, namely with a Roman figure to indicate the number of the series and an Arabic figure which is the patients individual number.

SEVENTH CHAPTER.

Technique and Errors of Measurements.

The present work deals with elliptocyte counts in so-called elliptocytics, in normal persons and in normal persons who have had injections of elliptocytic blood. It is necessary to measure the accuracy of the determinations in all of these cases. The factors which affect it are as follows:

1. The fact that the elliptocyte count in the blood in the three groups mentioned is subject to real changes, depending for example on the blood being taken under different circumstances and on variations in the charcteristics of the persons concerned.
2. Another cause of error is that the differences between elliptocytes and round cells may be assessed differently on different occasions. More elliptical cells may be reckoned as round on one occasion than on another. It is possible that the limit is set differently when elliptocytes are numerous than when they are scarce.
3. The standard deviation also depends on how many corpuscles are counted. From a purely theoretical point of view it may be stated that the error will diminish in proportion to the square root of the number counted. Thus if four times as many corpuscles are counted the error will be diminished by half.

There are various methods of approach to the estimation of the elliptocyte count. One may judge by eye which cells are elliptocytes and which normal corpuscles. One may further measure the diameters of the individual corpuscles and use the figures thus obtained for differentiating between the elliptocytes and the normal cells.

Definition and classification of elliptocytes.

Measurement of the eccentricity of elliptocytes must obviously be carried out by measurement of the longest and shortest diameters. The eccentricity as derived from these measurements, may be expressed in different ways where a = the longest diameter and b = the shortest diameter. Günther's expression for the eccentricity of the blood corpuscles, $\sqrt{1-\left(\frac{b}{a}\right)^2}$, appears unnecessarily complicated as a useful value for the eccentricity[1] can be obtained from the expression b/a. This value has therefore been used in the present work as the

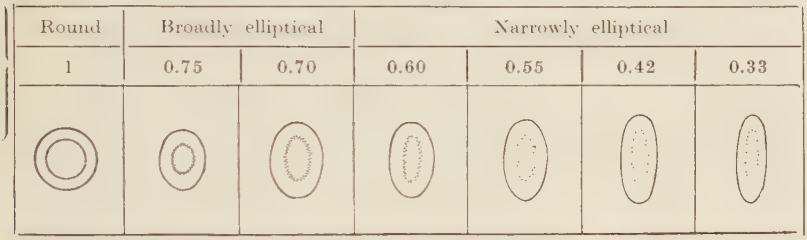


Fig. 1. Diagram showing red blood corpuscles in elliptocytic blood with different degrees of ellipticity.

expression of the ellipticity of the blood corpuscles where measurements have been made. It follows from this expression that the ratio between the two axes is 1 for circular corpuscles and the more elliptical the corpuscle is the nearer the value for the ellipticity approaches 0 (fig. 1).

The *measurements* were made with a Zeiss eyepiece micrometer using a Wratten C filter in blue light and using the largest possible numerical aperture. The measurements were always made at the same magnification (· 538). As no attempt was being made to determine the absolute diameter of the corpuscles the degree of magnification was of little importance. The blood was diluted 1/100 with Hayem's solution.

Measurements were not made on the actual material used for the research except in isolated instances. The method cannot in fact be considered for any comprehensive investigations as it is too time-consuming. The measurement

[1] When the eccentricity is as mentioned above (foot-note pag. 14), calculated from a given mathematical formula valid for the ellipse, the present author preferred to employ the notation "ellipticity" for the ratio between the smallest and the greatest axis.

of 500 cells takes almost a working day to perform. When about 3500 such measurements have to be performed the time-consuming nature of the method becomes obvious and important.

However, in order to check the accuracy of such measurements, they were carried out twice after different intervals of time on a normal person and on an elliptocytic. The results are given in table 6 a. The medians and quartiles for the measurements made are given in the table 6 b.

Table 6 a. Absolute and percentage distribution of red blood corpuscles according to the ratio between their axes in two cases, one with normal blood and one elliptocytic.

Ratio between the smallest and the greatest diam.	Normal blood				Elliptocytic blood			
	1st experiment		2nd experiment		1st experiment		2nd experiment	
	Number	%	Number	%	Number	%	Number	%
0.2—0.3	—	—	—	—	5	1.0	—	—
0.3—0.4	—	—	—	—	20	4.0	15	3.0
0.4—0.5	4	0.8	2	0.4	58	11.6	55	10.9
0.5—0.6	6	1.2	2	0.4	65	13.0	50	9.9
0.6—0.7	14	2.9	4	0.8	125	25.0	76	15.1
0.7—0.8	50	10.3	23	4.7	65	13.0	120	23.8
0.8—0.9	141	29.1	95	19.4	67	13.4	109	21.6
0.9—1.0	270	55.7	364	74.3	95	19.0	79	15.7
Total	485	100.0	490	100.0	500	100.0	504	100.0

Table 6 b. The ratio between the axes of the blood corpuscles expressed as median and quartiles in two cases, one with normal blood and one elliptocytic.

	Normal blood		Elliptocytic blood	
	1st experiment	2nd experiment	1st experiment	2nd experiment
No. of corpuscles ..	485	490	500	504
1st quartile	0.834	0.897	0.565	0.608
Median	0.910	0.933	0.682	0.747
3rd quartile	0.955	0.967	0.856	0.858

The figures show that the method is not completely exact. For the present purpose however there is no point in inquiring further into the degree of exactitude.

As has been mentioned above, it is possible to estimate by eye which cells are elliptocytes and which normal corpuscles. The accuracy of such estimations can, of course, only be determined empirically. It is obvious that if there are many cells on the borderline between what one is accustomed to regard as an ellipse and what one is accustomed to regard as a circle the error will be greater than if there are only a few cells on that borderline and most of the cells are extremely elliptical or perfectly circular.

Technique of withdrawal of blood and photography.

The blood for testing was usually drawn from the pulp of the finger with a needle. In babies it was often drawn from the heel. The blood was diluted with Hayem's solution usually in the proportion 1/100, which gave a suitable concentration for photography. The test material was set up in a specially constructed chamber. This was made of a microscope slide to which two narrow strips of coverslip glass had been fastened with paraffin about 4 cm apart and parallel with one another. Great importance was attached to the suspension of the corpuscles which should naturally be as homogeneous as possible. The blood specimen was then mixed for at least 10 minutes in an Enghoff's (1937) mixing apparatus.

A Leitz microscope with a microphoto attachment (made by Busch—Rathenow) was used for photography of the blood specimens. Obj. akromat 10 : 1. Ok. periplan 10 : 1. Magnification × 100. The system was focussed carefully as described by Köhler. A special strong microscope lamp of the Busch—Rathenow type was used with a blue filter. After the plates had been developed a further enlargement of about × 2 was carried out in an enlarging apparatus. The photographic field was divided into squares to facilitate calculations. This was done by projecting the image of a square ruled sheet of glass on to the printing paper. The plates measured 6 $^1/_2$ × 9 cm, the printing paper 13 × 18 cm (Agfa-Brovira extra hard). Satisfactory pictures have in general been achieved using this method. It was not possible to correct completely a certain amount of blurring of the edges due to spherical abberation. It may be that the use of another lens system might achieve technically better photographic results. However, the ideal equipment was unobtainable owing to wartime conditions (fig. 2).

About 2000—4000 cells were photographed on each print using the method of photography described above. It was, however, often necessary to exclude some of the peripheral squares from the calculations owing to the blurring of the edges mentioned above. Bad prints were rejected. Great importance was attached to allowing the corpuscles to settle for long enough (at least 10 min.) in the chamber before the photographs were taken. In this way it was generally possible to get the corpuscles in the same projection as they have a tendency to lie with their greatest surface on the slide. Thus errors due to oblique projection are, to a large extent avoided. Photographs were taken after shorter or longer periods of sedimentation in order to determine the importance of this factor for the accuracy of the method, and the changes in the elliptocyte count in one and the same preparation were determined (table 7).

Table 7. Elliptocyte count in the same sample of blood after different periods of sedimentation.

Period of sedimentation in min.	% Elliptocytes
4	1.76
6	1.74
8	1.77
14	1.72
16	1.79

The table shows that no definite difference can be demonstrated for periods greater than 4 minutes. Any further error caused by oblique sedimentation is included in the experimental error which is calculated below.

Technique of counting.

Counting of the cells on the print was carried out with the aid of a magnifying glass having a magnifying power of about × 3 with direct illumination. The counting was materially facilitated by the use of mechanical counters. The total number of cells was counted first, and then the number of elliptocytes. In carrying out counts from the prints an attempt was made only to include the definitely narrowly

elliptical forms which are found in a fairly high percentage in elliptocytic blood but which only appear as isolated occurences in normal blood. Measurements have been made on one and the same cell in order to ascertain what degree of ellipticity forms the most natural line of demarcation between elliptocytes and non-elliptocytes when making estimations by eye. The result is given in table 8 (cf. fig. 1).

Table 8. Comparison between estimation and measurement of the ellipticity of the corpuscles in elliptocytic blood.

The ratio between the smallest and the greatest diameter.

Shape of the corpuscles by estimation	Number of corpuscles having the ratio between the axes given below								Total	
	1—0.9	0.9—0.8	0.8—0.7	0.7—0.6	0.6—0.5	0.5—0.4	0.4—0.3	0.3—0.2	Number	%
Normal round ...	29	22	29	—	—	—	—	—	80	20.0
Broadly elliptical.	1	11	61	96	9	—	—	—	178	44.5
Narrowly elliptical	—	—	1	11	44	60	20	6	142	35.5
Total	30	33	91	107	53	60	20	6	400	100.0

The table shows, as was to be expected, that it is for the most part cells with an index of ellipticity near to 1, i. e. between 1 and 0.7, which are estimated as round, while those with an index between 0.6 and 0.2 are usually estimated as narrowly elliptical. The table further shows that some cells, usually having an index between 0.8 and 0.6 are estimated as being broadly elliptical. These are regarded as normal in the estimations given below. It thus appears from the table if the limit for roundness is set at an index of 0.7 then 7.3 % of the cells are round according to measurement while 20 % are considered as round by estimation. Where there was doubt as to whether a cell should be considered as narrowly elliptical or broadly elliptical the length of the corpuscle was the descisive factor. Round corpuscles or slightly elliptical forms lying askew could usually be detected owing to their biconcavity which is absent, or only present to a very slight extent in the narrowly elliptical forms. If the corpuscles are lying askew so that their shapes may appear to be narrowly elliptical it is usually possible to decide whether one is dealing with an elliptocyte or not by

means of the length, this is considerably greater in elliptocytes than the diameter of the normal red corpuscle. The estimations were carried out by the author and an assistant (fig. 3 and 4).

Errors of estimation.

a. Coefficient of variation.

The main investigation into experimental errors was that in which the blood was investigated in the same way as the blood in the main material. The reliability of the estimation was determined by carrying out 20 determinations on specimens of blood having different elliptocyte counts. The determinations were carried out by the author and by his assistant using the same liquid. This investigation does not include errors arising in dilution or pipetting. These, however, are of little importance in this connection as the point being investigated is the relative elliptocyte count and not the absolute count. The standard deviations of different elliptocyte counts has been calculated from these determinations and the figures are given in table 9.

Table 9. Average percentage elliptocyte count in normal blood, elliptocytic blood and mixtures having different elliptocyte content. The determinations were carried out by two persons separately.

$M \pm \varepsilon(M)$ = mean ± standard error of the mean. σ = standard deviation.

Author's values				Assistant's values			
Number of determinations	$M \pm \varepsilon(M)$	σ	σ in per cent of M	Number of determinations	$M \pm \varepsilon(M)$	σ	σ in per cent of M
Normal blood							
19	0.062 ± 0.013	0.056	90.3	19	0.099 ± 0.015	0.067	67.7
Mixtures having different elliptocyte content							
20	0.516 ± 0.019	0.086	16.7	20	0.541 ± 0.015	0.065	12.0
20	1.559 ± 0.049	0.221	14.2	20	1.484 ± 0.039	0.176	11.9
20	1.348 ± 0.051	0.227	16.8	20	1.529 ± 0.040	0.181	11.8
20	2.250 ± 0.043	0.191	8.5	20	2.304 ± 0.046	0.204	8.9
20	25.67 ± 0.31	1.38	5.4	20	23.17 ± 0.37	1.64	7.1
Elliptocytic blood							
20	50.30 ± 0.61	2.74	5.4	20	45.31 ± 0.53	2.36	5.2

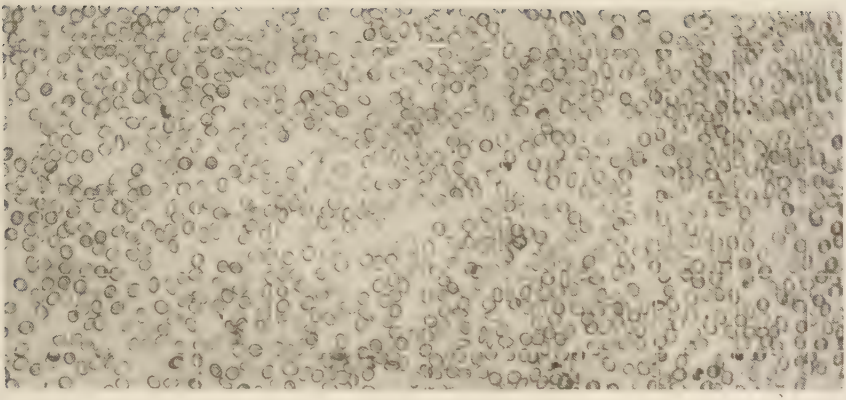


Fig. 2. The red blood corpuscles in an elliptocytic (case 10) with 27.3 per cent elliptocytes. The photographie field is divided into squares to facilitate calculations. Magnification about × 200.

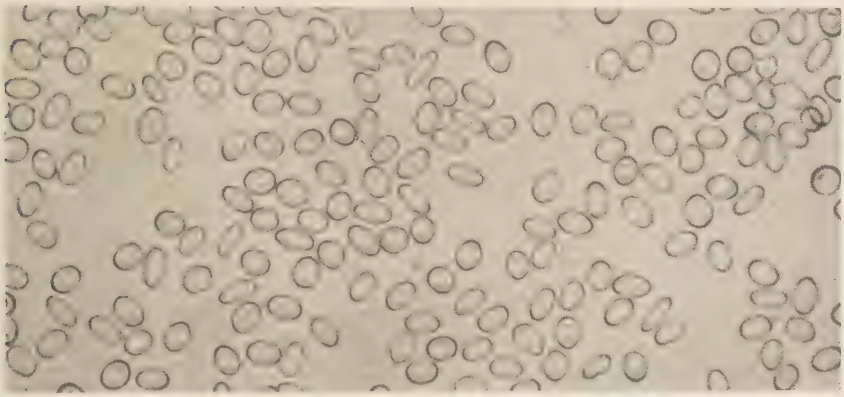


Fig. 3. Red blood corpuscles from another elliptocytic (case 8) with 27.0 per cent elliptocytes. Magnification about × 500.

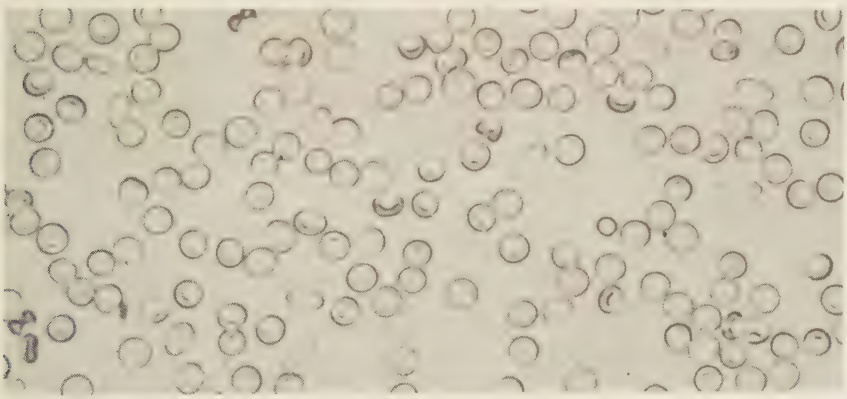


Fig. 4. Red blood corpuscles in a normal person with 2.3 per cent elliptocytes after intravenous transfusion with the elliptocytic blood seen in fig. 2. Magnification about × 500.

The first point shown by the table is that the figures obtained by the author and his assistant are in good agreement. It is further found that the absolute value of the standard deviation falls as the elliptocyte count falls, but the percentage value remains fairly

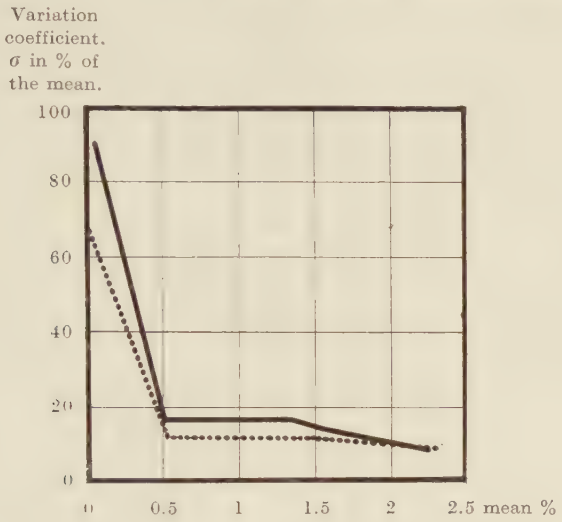


Fig. 5. Relationship between the average elliptocyte count at different dilutions and corresponding variation coefficient.

Continuous line = authors determination.
Dotted line = the assistants determination.

constant at a very low value. The *critical level* seems to be *where the elliptocyte count is about* $^1/_2$ %. Above this value *the experimental error remains about 10* %, but below it the error rises rapidly. This is illustrated graphically in fig. 5.

b. Comparison between theoretical and empirical values.

Information derived from a previous preliminary investigation on blood having different elliptocyte counts has been used to check the present determinations. The experiments were carried out by making mixtures of blood containing different amounts of elliptocytic blood and normal blood. It was thus possible to calculate a theoretical value for the elliptocyte count of the blood mixtures.

The author and his assistant then separately calculated the elliptocyte count in the usual way by counting the corpuscles on the same photograph

of the mixture. There were thus 2 empirical values for comparison with 2 theoretical values, as the author and his assistant each obtained a theoretical value based on experiments which they had carried out previously. The theoretical value is of course subject to an error which is ignored in this connection, or, more correctly, is included in the calculations. The difference between the theoretical and empirical values obtained by the author and between the theoretical and empirical values obtained by the assistant, were used for calculating the dispersion. It is obvious that these differences are correlated with one another as the same photograph was used for making the counts. This correlation, the magnitude of which is unknown, is of no importance as regards the value of the standard deviation obtained from the differences. On the other hand it is of importance in connection with the *accuracy of the standard deviation, i.e. its standard error.*

Table 10. Comparison between the empirically found and theoretically calculated percentage elliptocyte counts at different dilutions and the corresponding experimental errors.

d = difference between the empirical and theoretical values.

Elliptocyte count in %	Number of determinations	Average percentage elliptocyte count according to		$\sigma = \sqrt{\frac{\Sigma d^2}{n}}$	σ in % of M_3 $\left(M_3 = \frac{M_1 + M_2}{2}\right)$	$\frac{M_1}{M_2}$
		empirical determination $= M_1$	theoretical calculation $= M_2$			
<1	19 (13)	0.74	0.65	0.17	24.3	1.14
1—2	24 (16)	1.56	1.44	0.23	15.3	1.08
2—3	21 (11)	2.50	2.43	0.31	12.6	1.03
3—4	18 (10)	3.44	3.42	0.24	7.0	1.01
>4	66 (24)	9.94	9.88	0.93	9.4	1.01
Total	148 (74)	5.57	5.48	0.65	11.8	1.02

Table 10 therefore gives information both as to the determinations themselves and as to how many of them were carried out by the assistant. (The latter figures are given in brackets.) We have not calculated the standard error of σ. This information is included for the sake of completeness. It must be pointed out that the accuracy obtained by making single counts on different photographs is less than that obtained by making 2 counts according to the method used here. The accuracy achieved is not, however, as great as though completely separate determinations had been carried out. Thus, if one calculates on the basis of all the values, one gets too small

a standard error, while if one calculates on the basis, either of those experiments carried out by the author or of those carried out by his assistant, the value for the standard deviation is too large. The correct standard error lies somewhere between these values. It is not possible to decide where it lies without calculating the correlation which would be unduly difficult.

The table is, in fact, only intended as a further control. It shows that the value for the standard error calculated in this way remains at about the same level as that calculated on the basis of more correct data.

c. Variations with time.

It was pointed out at the beginning of this chapter that the elliptocyte count in the blood of an elliptocytic or of a normal person may be expected to vary depending on the occasion on which the blood is taken. Table 11 shows counts made on the same elliptocytic at different times.

Table 11. Elliptocytic blood from one and the same individual determined on 16 occasions during 1 year.

$M \pm \varepsilon (M)$ = mean ± standard error of the mean.
σ = standard deviation.

Investigated by	Number	$M \pm \varepsilon (M)$	σ	σ in % of M
Author	16	49.9±0.50	1.99	4.0
Assistant.....	16	44.0±0.51	2.00	4.5

The table shows that the scatter is not greater than that which might occur with counts on the same sample of blood (see table 9). It is obvious that the scatter must theoretically be greater than the standard error when the determinations are carried out over so long a period of time. The fact that the variation was not larger may be due to chance and have been caused by the smallness of the number of estimations made which was only 16. The mean values obtained by the author and the assistant show a significant difference (5.9± 0.71), the value obtained by the author being higher. The scatter is about the same in both investigations. (The material is only counted by the assistant.)

d. Errors arising in vivo.

It is theoretically possible that variations in the elliptocyte count are greater in a person who has received an injection of elliptocytic blood than it would be if the bloods were mixed in vitro, depending, for example, on abnormal mixing conditions (see tables 9 and 10). The author has therefore carried out some estimations of the standard error by means of double tests on the various determinations which were carried out on blood with different elliptocyte counts during the course of the research. Samples of blood were taken simultaneously from the right and left hands for this purpose (table 12).

Table 12. Error of measurement $\left(\sqrt{\frac{\Sigma d^2}{2n}}\right)$ of the elliptocyte count in vivo determined by double determinations.

d = difference between the elliptocyte count in 2 samples of blood from the same person.

Elliptocyte count in %	No of double determinations	Mean right hand $= M_1$	Mean left hand $= M_2$	Standard error of a single measurement	σ_i in % of M_3 $\left(M_3 = \frac{M_1 + M_2}{2}\right)$
< 1	10	0.46	0.46	(0.093)	(20.2)
1— 5	33	3.24	3.17	0.370	11.6
15—26	9	21.50	22.50	(1.331)	(6.1)
Total	52	5.86	5.99	0.629	10.6

The table shows that the value thus obtained for the experimental error agrees very well with that found by the method previously described.

e. Errors of mixing.

It is of some importance in this connection to try to get an idea of how fast mixing of elliptocytic and normal blood occurs, both in the larger vessels and in peripheral vessels (so-called capillary blood) when an attempt has been made to increase the blood flow through the capillaries by making them hyperaemic by warming (table 13).

The table shows that the concentration of elliptocytes is certainly somewhat higher in venous than in capillary blood 20 minutes after the injection, but that the difference cannot be considered to be of

Table 13. Comparison between the elliptocyte count in venous blood and in capillary blood with and without previous induction of hyperaemia.

Time after transfusion	Capillary blood		Venous blood
	Heat not applied	Heat applied	
20 min.	2.98	2.78	3.04
1 hour 20 min.....	3.12	3.11	2.98
3 » 20 »	2.92	2.91	2.78
4 » 50 »	2.51	2.65	2.95
6 » 20 »	2.58	2.63	2.61
15 » 20 »	2.57	2.64	2.63

proved significance owing to the experimental error, and in any case it should not be of great importance. It was impossible to discover any definite difference between capillary blood with or without the application of heat.

EIGHTH CHAPTER.

Other Techniques Used.

Other techniques which have been used in this research are those for *haemoglobin* determination, *haematocrit* determination, counting of *red blood corpuscles* and determination of *osmotic resistance*. Determinations involving the use of *radioactive phosphorus* have also been made.

Haemoglobin.

Most of the haemoglobin determinations were carried out on capillary blood (from the pulp of the finger or the heel). In only 2 cases were the determinations carried out on venous blood. When the fingers were pricked the blood was made to flow freely and stasis was avoided as far as possible in venepuncture. Enghoff (1937) states that there is no systematic difference between the haemoglobin content of blood obtained in these ways after the first month of life.

Haemoglobin determinations were carried out by reading the extinction in a Pulfrich Stufenphotometer of the haemoglobin which

had been converted to alkaline haematin. The determinations were carried out on a layer 1 cm thick and filters S 53 was used. Three readings were taken for every determination.

The haemoglobin was standardised by Dr. Enghoff three times during the course of the experiments.

Möller and Vahlquist (1946) state that the normal haemoglobin content of the blood of children over 6 months old is 12.0—12.5 g% and that the mean value for the red cell count is 4.5 mill./mm^3. If the value of 15.3 g%, which corresponds with an oxygen capacity of 20.5 vol%, is taken as 100 % (Enghoff) the relative values for the haemoglobin content will be 78—82 % and the colour index 0.91—0.94. The normal value for the red cell count used here is 5.2 mill./mm^3 (Enghoff). It is more difficult to give minimum physiological values and they probably vary considerably from case to case (Möller and Vahlqvist). The lowest values found by these authors among children treated thoroughly with iron were however hb 9.7 g% (63.4 %), red cells 3.8 mill./mm^3, colour index 0.87.

Haematocrit.

Haematocrit investigations were carried out with Enghoff's haematocrit, taking the mean value for 2 double determinations. According to Enghoff the error introduced by using capillary blood for the determinations amounts to 1—2 %.

Red blood corpuscles.

The counting of the corpuscles was done on at least 2 separate samples of blood. Ellerman's method was used for the dilution of the blood, using Hayem's solution as the diluent. A mixing apparatus constructed by Enghoff was used to obtain homogenity. A Zeiss counting chamber, as designed by Bürger, was used for the counting. The chamber was checked by Enghoff. 100 small squares (0.0025 mm^2) were counted for each sample. According to Enghoff the error of the corpuscle counts amounts to about 2 %.

Osmotic resistance.

These were carried out according to the method of Dacie and Vaughan (1938). According to these authors the mean of the median values in 50 normal persons (arithmetic mean of median corpuscular fragility) was 0.366 ± 0.0015, $\sigma = 0.0105$. The limits of variation for the median were thus $M \pm 3\sigma = 0.33 - 0.40$ g% NaCl. The concentration at which haemolysis first occurred was stated to be 0.40—

0.44 g% NaCl. The authors state that it is hard to give a figure for the completion of haemolysis using this technique. According to Creed (1938) who uses a very similar technique, the onset of haemolysis is so gradual that he considers it impossible to give a value for the points at which it begins and is complete. The concentration of the salt solution which haemolysis 4 % of the corpuscles is given by him as 0.41—0.45 g% NaCl and the concentration which haemolyses 50 % is given as 0.36—0.40 g% NaCl.

Determinations of phosphate and radioactivity.

The total phosphate was determined by a modification of Briggs' method (Briggs 1922, see also Lindberg 1946). The radioactivity was determined by a method elaborated by Hevesy and his co-workers (see also Borell and Örström 1945). The values are expressed in terms of specific activity. This implies that the number of impulses per unit weight of total phosphate is calculated. Specific activity = $\frac{\text{number of impulses}}{\mu \text{ g total P}}$ [1].

NINTH CHAPTER.

Analysis of the Properties of the Blood of Elliptocytics.

Ellipticity in normal persons and in elliptocytics.

Previous authors have usually merely differentiated between elliptocytics and normal persons without determining whether limiting values exist, and without a detailed investigation of the morphological distribution of the corpuscles in both groups. However, if one is to get a better characterisation of the condition one must investigate the occurence of elliptocytes in both sorts of subject. The author first investigated 10 normal persons. Table 14 shows the distribution of the corpuscles in respect to their deviation from roundness, and also the median and quartiles. Fig. 6 and 7 are also given as illustrations of this distribution. They show that the majority of the corpuscles are round but a few show a moderate ellipticity. The

[1] The determinations of phosphate and radioactivity were entirely carried out by Docent Örström of the Wenner-Gren Institute, Stockholm.

Table 14. Percentage distribution of the red corpuscles in 10 normal persons according to the ratio between their two axes.

Normal persons	The ratio between the axes						First quartile	Median	Third quartile
	1—0.9	0.9—0.8	0.8—0.7	0.7—0.6	0.6—0.5	0.5—0.4			
A	73.6	17.2	7.4	1.6	0.2		0.892	0.932	0.966
B	74.3	19.4	4.7	0.8	0.4	0.4	0.897	0.933	0.967
C	80.4	16.1	3.1	0.4			0.907	0.938	0.969
D	76.8	17.8	5.0	0.4			0.902	0,935	0.967
E	72.2	20.4	5.4	1.8	0.2		0.886	0.931	0.966
F	84.2	11.4	3.2	0.4	0.4	0.4	0.911	0.941	0.970
G	82.4	13.4	3.8	0.4			0.909	0.939	0.970
H	71.8	19.4	7.0	1.0	0.2	0.6	0.884	0.930	0.965
I	73.6	18.0	6.4	1.6	0.4		0.892	0.932	0.966
J	82.8	13.2	3.0	0.4	0.6		0.909	0.940	0.970

ratio between their axes seldom falls below 0.5. Corpuscles with an ellipticity of 0.7 are rare.

The same investigation was carried out on 10 elliptocytics. The distribution of their corpuscles is shown in table 15 and is also illustrated by two figures (Fig. 8 and 9). It is noteworthy that the distribution is very nearly the same in all of these subjects although many are not related to one another. The simularity between people who are not related is actually about as great as that between those who are. A larger series would naturally be required to determine whether the interfamilial variability is noticeably greater than the intrafamilial.

In p. 60, table 6 a and 6 b the author has given the values obtained by duplicate estimations on one and the same subject. It appear from the figures in table 15, that the difference between one elliptocytic and another should hardly exceed the standard error of the method of measurement. The position is further clarified by the medians and quartiles for the 20 cases investigated (table 14 and 15). The difference between normal subjects and elliptocytics is seen, in general, to be very marked, and there can be no doubt as to the diagnosis. The position is, in fact, that normal subjects have no significant numbers of corpuscles having an ellipticity of < 0.7 while a large proportion of the corpuscles of ellip-

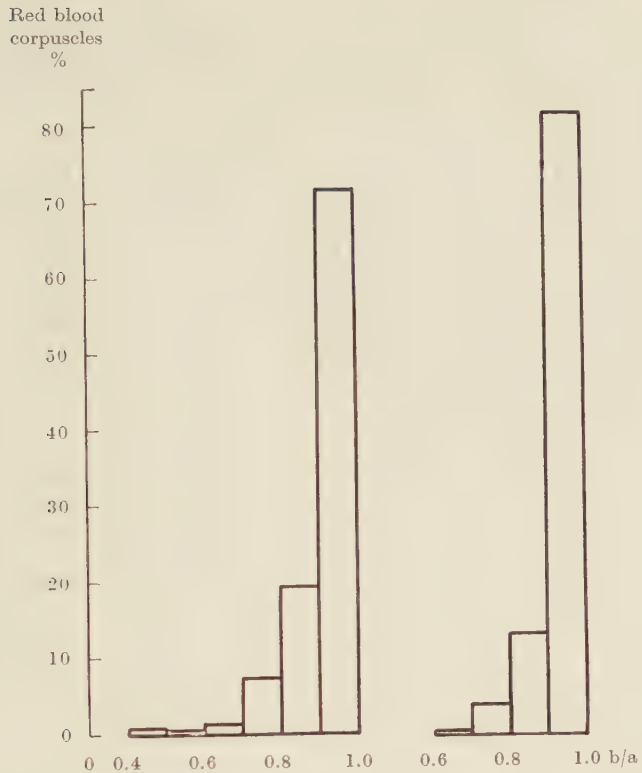


Fig. 6 and 7. Distribution of the red blood corpuscles in normal persons as a function of the ratio between their axes (b/a).

The highest elliptocyte content observed to the left, the lowest elliptocyte content to the right.

tocytics have lower ratio between their axes. This is true for the whole series.

Table 16 gives the percentage elliptocyte count for several elliptocytics. The table also gives a comparison between the values of the ratio between the axes (b/a) found by measurement and those obtained by estimation (% elliptocytes). By graphic interpolation along the summation curves constructed from the values given in the table 15 (see fig. 10) one can obtain the highest value for b/a corresponding with the percentage value for the elliptocyte count obtained by estimation. The figures for the highest value for b/a are seen to be very similar as between one elliptocytic and another. It is also seen that the average value for the ratio between the axes obtained by

Table 15. Percentage distribution of the red corpuscles in 10 elliptocytics according to the ratio between their two axes.

Elliptocytics	The ratio between the axes 1—0.9	0.9—0.8	0.8—0.7	0.7—0.6	0.6—0.5	0.5—0.4	0.4—0.3	0.3—0.2	First quartile	Median	Third quartile
1.....	26.0	9.2	20.6	21.0	9.6	8.4	4.0	1.2	0.609	0.728	0.904
2.....	19.8	24.4	15.2	17.6	12.2	7.6	2.8	0.4	0.611	0.762	0.879
3.....	22.4	17.6	23.2	11.2	7.6	12.0	4.0	1.8	0.592	0.757	0.885
4.....	24.0	21.2	14.4	19.8	11.4	5.6	3.4	0.2	0.622	0.767	0.895
5.....	20.0	15.0	30.2	23.8	5.6	4.6	0.8		0.659	0.750	0.867
6.....	21.0	14.6	27.2	15.8	7.2	9.0	3.4	1.8	0.623	0.747	0.873
7.....	24.2	11.0	26.0	18.0	9.0	10.0	1.6	0.2	0.623	0.743	0.893
8.....	14.4	26.0	21.4	16.6	11.8	8.8	0.8	0.2	0.620	0.755	0.859
9.....	5.6	9.4	18.8	26.1	21.9	15.4	2.8		0.531	0.638	0.747
10.....	19.0	13.4	13.0	25.0	13.0	11.6	4.0	1.0	0.565	0.682	0.856

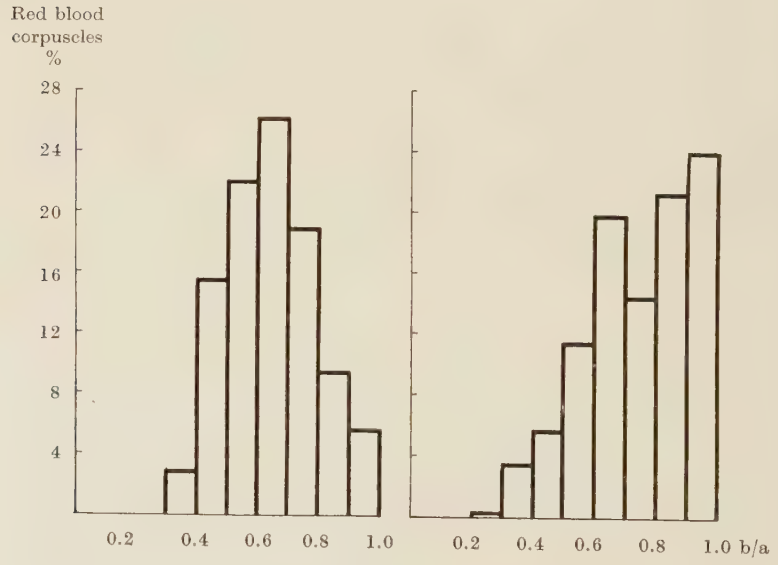


Fig. 8 and 9. Distribution of the red blood corpuscles in elliptocytics as a function of the ratio between their axes (b/a).

The highest elliptocyte content observed to the left, the lowest elliptocyte content to the right.

Table 16. Maximum value of axial ratio (b/a) in red blood corpuscles counted as elliptocytes in 10 elliptocytics; the percentage of elliptocytes in all cases is obtained by estimation method.

Elipto-cytic	Number of estima-tions	Average percentage of elliptocytes obtained by estimation	As elliptocytes are counted all red blood corpuscles with a lower value of b/a than:	Haemo-globin %	Number of red corpuscles per mm^3 (millions)
1	9	21.7	0.57	85	4.80
2	6	26.1	0.62	80	4.06
3	4	20.8	0.54	91	4.54
4	1	16.3	0.56	87	4.20
5	1	12.8	0.61	85	4.90
6	5	23.2	0.61	85	4.18
7	2	19.5	0.59	86	3.89
8	6	27.0	0.63	85	4.95
9	16	44.0	0.61	77	3.47
10	6	27.3	0.57	88	5.19
Mean			0.59		

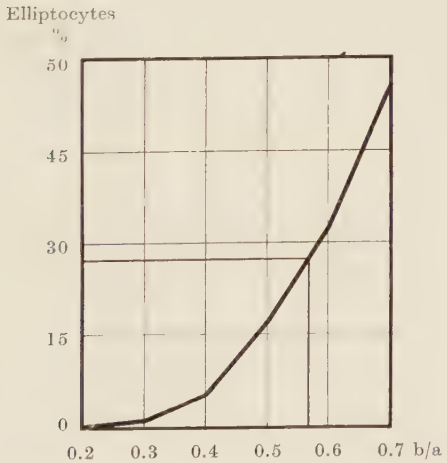


Fig. 10. Diagram showing graphical interpolation of the maximal axial ratio (b/a) corresponding to a given elliptocyte count as found by estimation.

interpolation (0.59) corresponds quite well with the value for the ellipticity for the narrowly elliptical forms found in table 8, p. 63, i. e. 0.6. The table 16 also shows the values for the haemoglobin content and the red cell count which show that elliptocytics are not anaemic.

There is a minor point which must be mentioned in this connection, namely that of oblique projection causing apparent ellipticity. This has been treated briefly in the chapter 7. The ratio between the axes thus produced is the cosine of the angle between the corpuscle and the horizontal plane. It is thus clear that an ellipticity of 0.7 corresponds to an angle of 45°. It should be easy to detect such a turning under the microscope as it would lead to a clearly measurable difference in level between the top and bottom of the corpuscle. Such differences were not observed and this ought to provide a further reason for not taking this source of error into account.

Constancy of the elliptocyte content.

It is a question of some practical importance as to how constant the properties are. Most authors have assumed them to be constant without making any proper investigations as to whether they were. They did, of course, make observations at intervals and found no differences. The present author has also carried out repeated determinations on the same individual at intervals, making up to 16 determinations over one year. They showed no difference worthy of note. It therefore seems unnecessary to go further into this question. The reader is referred to table 11, p. 67 for further information.

Elliptocytics and disease.

As it has been suggested that elliptocytics are specially prone to haemolytic anaemia and other disorders it must be mentioned that the 10 elliptocytics investigated by the author presented no abnormalities in these respects. None showed signs of anaemia. The fact that one of them had a haemoglobin value of as low as 67 % on one occasion was probably due to his having recently been bled (see table 16).

Resistance of elliptocytes.

Furthermore the author determined the osmotic resistance of the corpuscles of several of the elliptocytics by the method of Dacie

Table 17. Osmotic resistance of the red blood corpuscles from 9 elliptocytics in NaCl solutions (g %).

Elliptocytics	25 % of the corpuscles haemolysed (first quartile)	Median corpuscular fragility (Median)	75 % of the corpuscles haemolysed (third quartile)
1	0.390	0.374	0.360
2	0.385	0.371	0.361
3	0.414	0.395	0.373
4	0.400	0.373	0.360
5	0.370	0.354	0.340
6	0.399	0.389	0.371
7	0.400	0.378	0.367
8	0.411	0.393	0.381
9	0.417	0.398	0.387
Mean	0.398	0.381	0.367

and Vaughan (see p. 70). The various median values for the fragility which are given in table 17 lie within normal limits as defined by these authors (0.334 – 0.398 g% NaCl). Comparison with the mean value (0.366 g% NaCl) given by the authors above shows that the values obtained in 8 of the present cases lie above it and only one below. It is thus possible that a larger series would show that haemolysis occurs somewhat earlier in elliptocytic blood than in normal blood. As the values for the beginning and completion of haemolysis are somewhat unreliable the author has used the values for the first and third quartiles instead.

It is easy to observe under the microscope that elliptocytes are haemolysed in the same way as ordinary corpuscles, i. e. they become completely spherical before they burst and can no longer be distinguished from round corpuscles.

This experiment confirms the results of most previous workers, namely that *the red blood corpuscles of elliptocytic blood show no lowering of osmotic resistance*. The present author was unable to demonstrate that they have a raised resistance as has been maintained by some authors. It is possible that these authors investigated abnormal cases.

Aggregation of elliptocytes.

The aggregation of elliptocytes seems to occur in the same degree as that of normal corpuscles but of course the clumps are not the regular rouleaux which are characteristic of normal blood. The sedimentation rate was normal in the 9 elliptocytics in whom it was investigated.

It is also of interest to know to what extent elliptocytes aggregate with normal corpuscles in mixtures of elliptocytic and normal blood. In order to clarify this point the author made up mixtures each containing one part of elliptocytic blood and 10 –20 parts of either blood with a high sedimentation rate or blood with a low sedimentation rate. The blood was well mixed and the sedimentation rates of the two mixtures determined. Two such experiments were carried out. Samples were then taken from the original mixtures and from the corpuscular layer after a certain degree of sedimentation had taken place. Photographs were then taken in the usual way for the determination of the elliptocyte counts in the different samples. Two further experiments were made using mixtures containing half elliptocytic blood and half normal blood or blood with a high sedimentation rate. The results are given in table 18.

Table 18. Elliptocyte count in a mixture of elliptocytic and normal blood and in the upper layer of the column of corpuscles obtained by sedimentation.

	Percentage of elliptocytes			
Test on the original mixture	3.90	9.42	20.48	25.58
Test on the upper layer of the blood column after sedimentation	3.24	9.78	19.80	24.73

The table shows that the elliptocyte count in the upper layer of the blood column is the same as that in the original mixture within the limits of the error of measurement. This implies that the elliptocytes do not remain in the upper layer but are incorporated normally in the rouleaux. As Fåhraeus in particular pointed out, rouleau formation which occurs in vivo and is an intravital phenomenon when pronounced, influences the distribution of the corpuscles not only in the cross-section of the vessels of finer calibre, but also

in the various parts of the vascular system as a whole. *This experiment thus suggests that one may count on elliptocytes being uniformly distributed among the normal corpuscles after transfusions.* Table 12 and 13, chapter 7 where double determinations were carried out also provides evidence for this.

TENTH CHAPTER.

Technique of Transfusion of Elliptocytic Blood.

Intravenous transfusions.

The blood of the elliptocytic was usually taken immediately before the transfusion. It was collected in a sterile glass vessel containing a small amount of heparin in about 10 cc of physiological saline. (The haematocrit value was, of course, determined on this mixture and not on blood taken directly from the donor.) In some cases (VII_1; VII_2; VII_3; VII_4; VII_5; VII_6) bottled blood was used. This was never kept for more than one day before transfusion and was stored in sterile mineral water bottles. Nothing further was added to the blood which was kept. In 3 of the cases (X_3; X_4; XII_1) the plasma was removed before transfusion by washing 3 times with Ringer's solution and centrifuging for 20 minutes each time. The plasma which had been removed was replaced with an equal amount of sterile Ringer's solution. (According to Wiener, 1946, and Ross and Chapin, 1943, and other authors, one day's storage appears not to have any influence on the lifetime of the red corpuscles.) In most of the cases the blood groups of the donors and recipients were the same, in the rest of the cases donors of group 0 were used as universal donors. All the donors were Rh-positive. The donor belonging to group 0 who was most frequently used has a low agglutinin titre.

The amount of blood transfused was, on the average about 13 % of the recipient's blood volume. (In chapter 12 this was calculated as being 7.4 % of the body weight.) The transfusions were carried out either by exposing and cannulating the vein or through a needle inserted intravenously. The blood had to be transfused fairly quickly. The transfusion took, in general, about 10 minutes. No complications whatever were observed.

Intraperitoneal transfusions.

The blood of the elliptocytics was taken in the same way as for intravenous transfusion, but washed or stored blood was not used. A large proportion of the donors belonged to group 0 and were used as universal donors regardless of the blood group of the recipients. The amount of blood transfused was, on the average, about 13 % of the calculated value for recipients blood volume. The injection was made into the left iliac fossa or half way between the umbilicus and the symphysis pubis. The needle used was fairly long, of medium bore and not ground to too sharp a point. The transfusion was carried out by injection and special care was taken to make the injection of the blood already at the time when the needle passed through the peritoneum in order to avoid perforation of the intestine. (In one case, where the patient already had extreme meteorism, the blood may have been injected into the intestine. The blood was not resorbed into the blood circulation. The patient developed vomiting and melaena but this cleared up quickly.) When the amount of blood injected was so large that it amounted to more than 20 % of the calculated blood-volume the abdomen was quite tense after the injection but it gradually softened within 24 hours as the resorption proceeded. The children also vomited a little sometimes after large injections. These initial troubles connected with the actual injection of the blood only occurred in exceptional cases, and apart from this the children benefited from the transfusions in the long run.

ELEVENTH CHAPTER.

Destruction of Red Blood Corpuscles after Transfusion.

Theoretical discussion.

It should be possible to obtain some information as to which of the postulated mechanisms of blood corpuscle destruction actually operates in the case of blood transferred by transfusion. Discussions of this subject have been concerned with two alternatives put forward by Schiodt. One is that the corpuscles are destroyed by the spleen irrespective of their age. The transfused corpuscles should disappear

according to an exponential curve. Schiodt calls this *the theory of destruction*. The other is that the corpuscles die of old age. As may be seen from the review of the literature Schiodt maintains that the transfused corpuscles would disappear linearly in this case. He calls this *the theory of longevity*.

I approached Professor Gunnar Dahlberg in connection with the analysis of these experimental results. He was kind enough to allow me to make use of an unpublished paper on the theory of the destruction of the red blood corpuscles. I wish to thank him for his kindness in this respect.

Dahlberg points out that if the corpuscles die of old age but nevertheless have different lifetimes the plot obtained may, in practice, be the same as that which would be obtained if they were destroyed at random by the spleen. Dahlberg refers to the latter case as *forcible destruction* and to the former as *inherent destruction*. These names give a better indication of the assumptions being made. It is thus impossible to prove the theory of the destructive activity of the spleen by means of transfusion experiments. It would, however, be possible to confirm the theory of inherent destruction if the lifetime of the blood corpuscles was fairly uniform. In that case one would obtain a straight line in plotting the rate of disappearance of the red corpuscles. It seems improbable, however, that the lifetimes of the corpuscles are so exactly uniform that there is no difference between them. One must almost always allow for some variability when dealing with biological phenomena. It thus seems to be impossible to differentiate between the two mechanisms by means of transfusion experiments.

The reader is referred to fig. 11 (taken from Dahlberg's paper) for an illustration of his argument. This was also suggested by Schiodt though he did not follow up the consequence of the theoretical possibility. The figure shows that the plot will take the form of a straight line until the time when the destruction begins to have an effect, it then falls off.

Dahlberg points out that the curves should be interpreted differently when making estimations of the lifetime of the red corpuscles but we need not go into that matter here.

Even if the problem of the destruction of the red blood corpuscles cannot be solved by means of the transfusion experiments carried out in this research it is of value from the point of view of further

Table 19 a. Percentage elliptocyte content in different normal subjects after *intravenous* transfusion of elliptocytic blood.

Time, hours	Case																		
	IV_2	IV_8	V_2	V_3	VI_3	VI_4	VII_1	VII_2	VII_3	VII_4	VII_5	$VIII_6$	IX_2	IX_3	IX_4	X_1	X_3	X_4	XI_2
Before transfusions	1.04	0.08	0.03	0.19	1.20	0	0.05	0.04	0.34	0.54	0.37	0.14	0.23	0.09	0.43	0.22	0.47	0.06	0.25
0.25 after transfusion	2.96	3.16	3.47			4.32	3.89	5.80	2.89	5.77	7.61		2.77	2.64	5.95	3.58	5.64	2.93	
0.30 » »																			2.93
0.50 » »					6.30														
1.00 » »																3.86			
1.10 » »																	6.35		
1.30 » »																			3.07
3.25 » »											7.84								
3.30 » »																			2.87
3.50 » »										6.57									
4.00 » »									2.86								6.08		
4.10 » »																2.79		3.25	
4.25 » »								5.94											
4.50 » »							4.31												
4.80 » »																			2.70
5.00 » »					4.25														
5.30 » »																		3.01	

6.00	»	»																3.37			
6.10	»	»																	6.09		
6.30	»	»																			2.61
7.00	»	»			3.76																
8.00	»	»					4.34														
8.80	»	»						5.31													
15.20	»	»																			2.61
15.40	»	»															3.08				
15.80	»	»														3.32					
16.10	»	»																5.87			
17.80	»	»																		2.76	
18.25	»	»																	5.87		
18.60	»	»																3.04			
20,50	»	»											5.33								
20.75	»	»			2.53	3.12						6.29									
21.00	»	»									3.18										
21.25	»	»	2.44																		
21.50	»	»								5.86											
21.75	»	»							4.12												
22.20	»	»																			2.56
22.25	»	»						4.23													
22.50	»	»					4.46														
24.00	»	.		3.37										3.80							

Table 19 b. Percentage elliptocyte content in different normal subjects after *intravenous* transfusion of elliptocytic blood.

Day after transfusion	Case																	
	IV_2	IV_8	V_2	V_3	VI_3	VI_4	VII_1	VII_2	VII_3	VII_4	VII_5	$VIII_6$	IX_2	IX_3	IX_4	X_1	X_3	X_4	XI_2
2	2.64	2.32	2.49	2.97	3.61	4.74	3.98	5.56	2.86	5.23	4.28	3.39	2.91	2.74	5.82	3.02	5.69	2.35	2.44
2.5	2.55																		
3	2.80	2.98	2.17	2.52	4.75	5.67	3.94	4.67	2.57	5.48	3.66	2.65	1.95	2.33	4.47	2.83	5.67	2.34	2.54
4	2.32	1.99	2.05		4.77	2.37	2.61	4.14	2.40	5.21	4.27	2.66	2.82	2.34	4.68	3.33	4.09	2.04	2.54
5		2.31	1.64	2.48			2.98	3.82	2.23	4.23	3.50	2.05				2.77	4.03	1.88	2.10
6		0.74	1.63	2.00	4.39	2.59	2.81	3.69	1.76	4.26	3.30		2.68	2.28	3.89	2.63	3.92	1.47	1.92
7	2.26	0.94	1.55	2.49	4.23	3.54	2.56	3.32	1.24	4.23	3.40	1.99				2.60	3.72	1.66	1.39
8	2.06						2.80	3.30	1.58	4.21	2.64	1.72	2.25	1.66	4.09	2.29	3.96	1.58	1.75
9		1.98	1.25	1.48	4.14	3.84	1.98		1.63	3.35	2.65	1.89				2.27	3.94	1.38	
10	1.40							3.26				1.78	2.22	1.09	3.42				1.93
11		1.19	1.58									1.78				2.26	3.11	1.40	
12					3.51	3.52	1.27	2.92	1.39	4.41	2.52								1.83
13		0.28														2.06	3.15	1.43	
14	1.36		1.22		3.49	2.24		2.61	1.44	2.12	2.16	1.09	1.53	0.41	2.07				1.82
15																1.93	2.39	1.18	

16		1.01	1.22		3.09	1.98	0.84	2.06	1.12	2.40	1.40								
17	1.45												1.49	0.20	2.71	1.68		0.59	1.54
18		0.95	0.84									1.05							
19	1.39				3.11	2.40	1.19	1.89	1.05	2.61	1.38					2.43	1.83	0.73	1.63
20		0.32	0.78																
21	1.63				3.57	2.00						1.06							1.12
22							0.88	1.33	1.13	2.02	1.20		0.51	0.15	2.17				
23		0.38			2.52	1.80						1.05				1.22	1.62		1.49
24	2.00		0.67				0.65	1.38	0.90	2.03	0.99		0.63	0.07	2.01				
25		0.42														1.02	1.72		
26					2.56	1.70													0.97
27		0.28					0.67	1.28	0.86	1.99	0.87		0.80	0.03	1.93	1.14	1.56		
28			0.86		2.51	1.63													
29							0.74	0.88	1.12	1.53	0.98					1.16	1.72		
30					1.83	1.52													
31																0.83	1.68		
32													0,44		0.57				
33					1.85	1.33			0.74	1.71	0.59	0.96							1.17
35					1.31	1.10													
37					1.16	0.84			0.50										
40					0.87	0.94													
41							0.32	0.36		0.71	0.49		0.22						

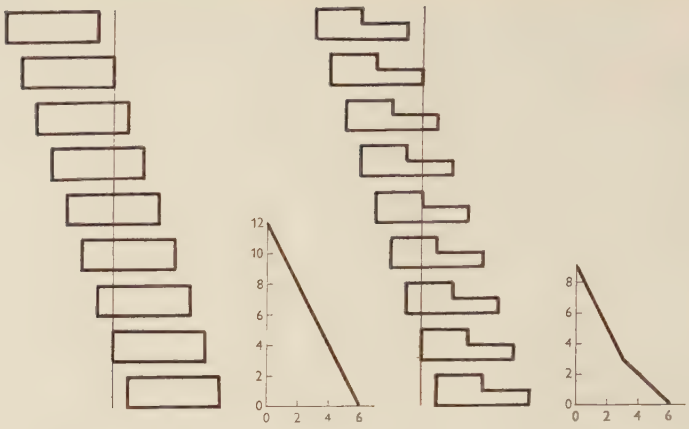


Fig. 11. A scheme illustrating the destruction of red blood corpuscles after transfusion. (according to Dahlberg)

The squares above show the blood corpuscles which are produced during different days. To the left they are supposed all to live for six days; to the right they are supposed half to live for three days and half for six days. At transfusion blood corpuscles produced during six days are included; they are of different age and, consequently, they die in the way the curves show.

investigations to have a rough empirical idea of how it takes place. *It seems most suitable to use an exponential function for smoothing the experimental data.* If inherent destruction should be the mechanism at work it is very unlikely that the variability in the lifetimes of the corpuscles will be very slight. It is only in such a case that the plot would be expected to have the form of a straight line.

The author has carried out 18 transfusions of elliptocytic blood on children between the ages of about 1 and 12 years and 2 adults. 5 of the children received 2 transfusions at intervals of some months. Some of the children had a low haemoglobin concentration and 3 of them had secondary anaemia (see table 5, p. 56). In 5 of the cases bottled blood was used and in 3 of them washed corpuscles. Table 19 a and 19 b, p. 82 gives an uncorrected synopsis of the whole series.

The times given for the drawing of the samples for testing may be considered as correct to within 5 minutes. The time at which the blood was taken on the day after the transfusion was, in most cases, between 20 and 24 hours after the transfusion, but there were a few cases in which it was taken between 15 and 20 hours after. In the following calculations the periods were treated as being ones of 24

hours although they sometimes differ in length by a couple of hours. These approximations would seem to be without consequence as the period of observation usually extended over several weeks. In series X and XI the times of withdrawal of samples are given correct to within 1 minute.

The author has assumed that the mixing of the corpuscles of the elliptocytic blood will occur within one or a few minutes. This agrees with the views of Nylin and of others (cf. the review of the literature p. 41) and furthermore the corpuscles of elliptocytic blood show the same conditions of mixing as ordinary blood corpuscles and seem to agglutinate normally with them in vitro (cf. p. 78). The author has therefore assumed that elliptocytes will also behave in the same way as round corpuscles in vivo. It has not, however, been possible to obtain any information as to the mixing conditions of the elliptocytes because, in most cases the withdrawal of blood samples did not take place either so frequently or at such accurately specified times as is required for a detailed study of these conditions.

Conditions of mixing.

In order to throw some light on the mixing conditions in vivo the author carried out more exact determinations in case XII_1 (table 20) both of the elliptocyte count and of the time of sampling. Four photographs were taken of each sample instead of the usual one and thus about 8000 - 9000 cells were available for counting. This should give an accuracy of about double that obtained in the rest of the cases. Blood samples were withdrawn from the beginning of the transfusion and for a sufficiently long time after it. The results appear in fig. 12 (cf. table 13 p. 69).

The table 20 and figure 12 seem to show that the mixing time of the elliptocytes is longer than that which has usually been found, for example, using corpuscles labelled with radioactive isotopes. It takes 15 minutes after the completion of the transfusion before the concentration of the transfused corpuscles reaches its maximum. The concentration then falls again and gradually reaches a relatively constant level. This peak in the curve may perhaps be due to mixing occurring first in the circulating blood and only later in the depot blood. Of course, it is also possible, though improbable, that the peak is due to chance. (Only very small amounts of blood were injected in the experiments of other authors using radioactive P).

Table 20. Percentage of elliptocytes in a normal person during and immediately after the transfusion of elliptocytic blood.

Total amount of blood transfused by the end of the time mentioned	Time, minutes	Elliptocyte count in %
	During transfusion	
	0	0.26
	1	0.37
100	5	0.77
200	8	1.63
475	10	2.21
	After transfusion	
	0	2.21
	5	2.95
	15	5.12
	20	4.23
	35	3.48
	50	3.44
	65	3.33
	365	3.71
	1140	3.63

The first observation on the large series was usually taken at least 15 minutes after the completion of the transfusion. This is designated as time 0. To judge by the investigation above one cannot rely on complete mixing of the blood having taken place within this period. Nevertheless the author did not consider it necessary to exclude this value when treating the curves mathematically (it is clear that it is a matter of indifference whether the first reading is included or not if enough observations had been made).

In fig. 13 the author has plotted two curves which were considered to be one of the best and one of the worst in order to obtain information as to whether an exponential curve corresponds with the observed values (see below for smoothing methods).

The whole series plotted on half-logarithmic paper shows that most of the plots give the impression of straight lines. There is, however, a large amount of irregularity in the course of the plots and one can naturally not exclude the possibility that some function other than

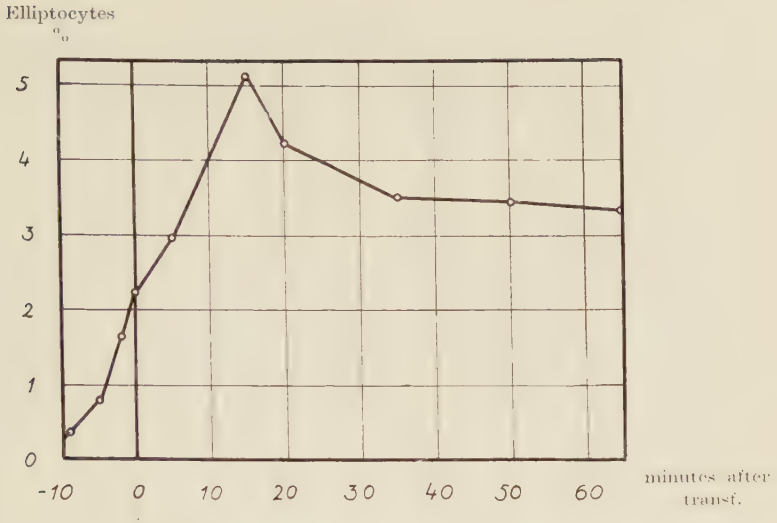


Fig. 12. The percentage elliptocyte content before, during and immediately after intravenous transfusion with elliptocytic blood.

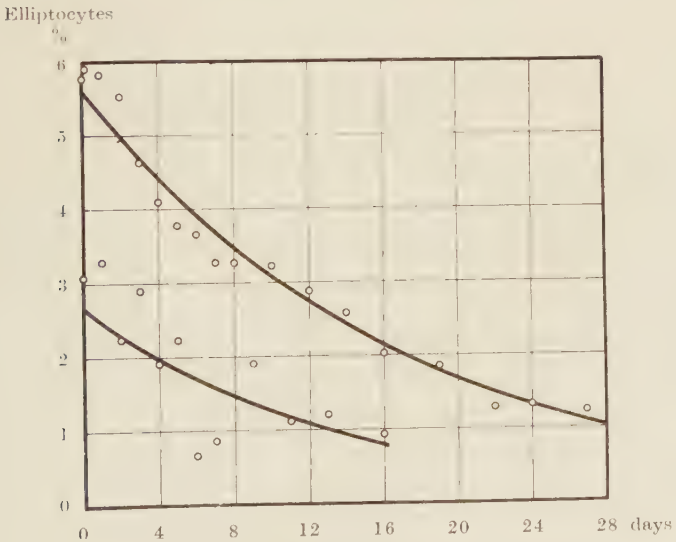


Fig. 13. One of the exponential curves best agreeing with the determinations and one of the curves with the worst agreement.

Smoothed with an exponential function and the method of least squares.

an exponential one would correspond equally well with the experimental values. We must remember what has already been pointed out by Dahlberg, namely that it is quite possible that another function which is only superficially similar to an exponential function is the correct one.

Corrections.

The elliptocyte count obtained by a direct estimation on the recipient's blood on all occasions after the transfusion must be diminished by the elliptocyte count he had before it if one is to obtain a correct value for the proportion of cells derived from the donor. The recipient always has a certain proportion of elliptocytes though it is usually very small. The present author has corrected this by simply subtracting the recipient's original elliptocyte count from each value of the elliptocyte count obtained after the transfusion. This is only an approximation as the proportion of the recipient's own elliptocytes in the total cell count diminishes a little with the increase in his blood volume due to the transfusion. Thus it would be theoretically correct to diminish the value of the elliptocyte count obtained directly, by a lower value for the recipient's elliptocyte count, and to increase the value as the recipient's blood volume diminishes to its original value. It is possible to make such a correction. It seems unlikely that this correction would be significant as the recipient's elliptocyte count is usually very low and the amount of blood transfused is but small. The calculations were carried out in a case where it appeared that these factors would have the greatest effect as the recipient had a relatively high elliptocyte count before the transfusion and the amount of blood transfused was also relatively large in comparison with the recipient's blood volume. It is not necessary to go into details of the calculations which are rather time-consuming, but it may be concluded that the correction has very little influence on the final result. Thus, in the case mentioned above earlier calculations using simple subtraction of the recipient's original elliptocyte count from the counts made after the transfusion gave a value of 4.73 % for the corrected elliptocyte count at time 0 and a half-life of 10.8 days while calculations using the proper correction (where different values for the recipient's elliptocyte count are subtracted from the direct count on different occasions, beginning with rather smaller values and later increasing slightly) gave values of 4.97 % and 11.2 days respectively. As this calculation was carried out on the case where it should have the greatest effect on the results *it was felt to be unnecessary to carry out this laborious correction for the recipient's elliptocyte count* and that *simple subtraction as described above would suffice.* As is shown in p. 65, the percentage error in estimation of elliptocytes rises markedly immediately the count falls below $^1/_2$ %. When making the calculations the author has therefore not used values of much below 1 %.

Smoothing processes.

As was shown earlier in this chapter it seems most suitable to smooth the observed values by using an exponential curve in order to obtain certain constants for the solution of other problems. The author has therefore chosen the simple exponential function $C_t = C_0 \cdot e^{-kt}$, where C_0 is the elliptocyte concentration at time 0, k is a constant and t is the time in days. If this function is converted to logarithms it gives the expression $\log C_t = \log C_0 - kt \cdot \log e$. This is seen to be a straight line when $\log C_t$ is plotted against time. The smoothing is done by the method of least squares using this equation. One thus obtains the constants C_0 and k which yield the equation of the curve which corresponds best with the observed values.

Half-life.

By aid of the "die away equation" just mentioned it is possible in every case to calculate the value for t when C_0 has diminished to half its original value, i. e. the *half-life* of the elliptocytes using the transformation $\textit{half-life} = \frac{\log 2}{k \cdot \log e} = \frac{0.69}{k}$. Table 21 shows the calculated values for k and the half-life and the mean value for these in all the experiments with its standard error.

The table shows that *the weighed mean value for the half-life is 13.1 ± 0.9 days* with a relatively large scatter among the individual half-lifes. A weighed mean was used in this connection because it is reasonable to assume that the differences between the values for half-life obtained for the separate individuals are due to error of measurements. Therefore the values for half-life, which are determined by more countings, should be given more weight than the others. The standard deviation for the half-life is just under 4 days (3.89 ± 0.64). Furthermore the distribution is very skew which may be due to chance as the material is relatively small.

A fairly rough empirical expression for the lifetime of the elliptocytes has thus been obtained by means of transfusions. Biologically speaking this means that *on an average half of the elliptocytes transfused into another person will live for more than 10—16 days.*

Case XII_1 was investigated more accurately and is also included in the larger material. The *half-life is here 15.3 days* if the values for the samples

Table 21. The value of the constant k and the half-life for different subjects. The mean of the figures for these 20 persons and the standard error are also given.

k = the constant in the function: $C_t = C_0 \cdot e^{-kt}$.

Case no.	No. of obs.	k	Half-life in days
VI_3	18	0.0353	19.6
XI_2	21	0.0375	18.5
VI_4	20	0.0379	18.3
X_1	19	0.0396	17.5
XII_1	34	0.0462	15.0
IX_2	10	0.0463	15.0
IX_4	13	0.0508	13.6
VII_4	20	0.0534	13.0
X_3	22	0.0568	12.2
VII_2	18	0.0603	11.5
IV_2	8	0.0628	11.0
$VIII_6$	14	0.0629	11.0
X_4	15	0.0661	10.5
V_2	13	0.0664	10.4
IV_8	12	0.0764	9.1
VII_3	13	0.0792	8.8
V_3	7	0.0845	8.2
VII_1	14	0.0864	8.0
IX_3	8	0.0911	7.6
VII_5	15	0.0950	7.3
Weighed mean		0.0578 ± 0.0039	13.1 ± 0.9

taken shortly after the transfusion are included in the calculation, and 15.0 days if they are excluded. There is thus no significant difference. The value for C_0 was also about the same by both methods. These values ought actually to be more reliable than those given above which are based on experiments which are not strictly comparable as the quantity of blood transfused did not amount to exactly the same percentage of the recipient's blood volume (see table 22 and fig. 14).

Before going further and using the values obtained it must be pointed out that the determinations are subject to certain sources of error in principle.

In the first place it might have been best to withdraw blood before transfusion. As things were it might be that the subject was somewhat overloaded with blood and that might to some extent affect the values. Further-

Table 22. Elliptocyte content in per cent after intravenous transfusion with elliptocytic blood in case XII_1.

Day after transfusion	Elliptocyte %	Day after transfusion	Elliptocyte %
0.8	3.27	15	1.73
2	3.05	16	1.45
3	2.80	17	1.35
4	2.71	18	1.14
5	1.88	19	1.96
6	2.48	20	1.43
7	2.16	21	1.21
8	2.77	22	1.21
9	2.22	23	1.29
10	2.16	24	1.18
11	2.61	25	0.96
12	2.05	26	0.93
13	2.29	27	0.91
14	2.02	—	—

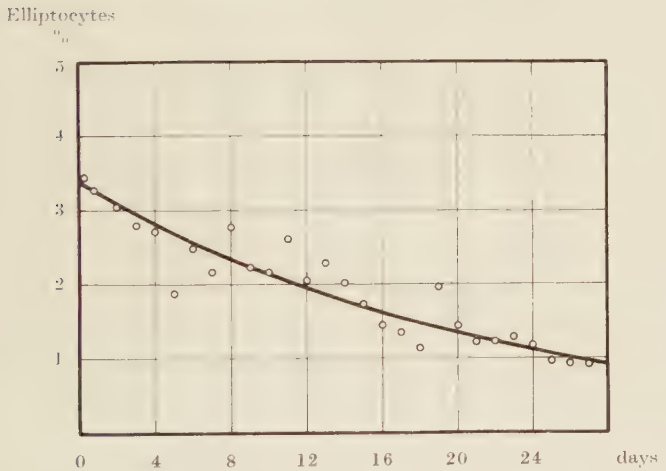


Fig. 14. The most accurately investigated intravenous curve (XII_1).
Smoothed with an exponential function using the method of least squares.

more one cannot exclude the possibility that elliptocytes have a lifetime different from ordinary red corpuscles. On the other hand the experiment (carried out by two workers) in which normal corpuscles were transfused into elliptocytics provides evidence that this is not the case (see p. 26 and 33). In the third place it may be that conditions are different in children than in adults.

It is impossible at present to make an accurate assessment of the significance of these sources of error. The material is quite unhomogeneous as it was impossible to use other patients as experimental subjects than those who were considered to require a blood transfusion. Some of the cases were therefore suffering from anaemia. The short period of conservation of the blood used in some of the cases and the washing of the corpuscles may perhaps also contribute to produce variations. Of course individual variations may also occur. It is thus quite natural that there should be a relatively high variability in the half-life. It has been my aim in this chapter to give a statement of the approximate value for the half-life. This question will be dealt with again in chapter 13.

TWELFTH CHAPTER.

Determination of the Blood Corpuscle Weight by the Use of Elliptocytes.

As was mentioned in the review of the literature, one can distinguish three fractions of the blood in the living subject, i. e.:

1. The blood circulating in the larger vessels and the finer capillaries.
2. The blood circulating in the paracapillary vessels and larger capillaries. This has a lower cell content than the first fraction.
3. The blood in the depots, specially the spleen which has a higher cell content than the first fraction.

The fractions are of different sizes under different physiological conditions so that it is hard to determine the blood volume exactly.

It is easier only to determine the corpuscle volume, but even so the facts just mentioned must be allowed for. It is specially important to be sure that the determinations are made after complete mixing with the depot blood. Opinions vary as to the time required for this. The author has himself carried out experiments which are described in an earlier chapter, p. 87. They indicate that the elliptocytes take a relatively long time, about 15 minutes, to mix with the whole of the blood at transfusion.

The author tried to use the information derived from the experiments described in the previous chapter for the determination of the

Table 23. The necessary constants for determining the weight of the red blood corpuscles.

Number	Weight kg	The amount of blood transfused in cm^3 (V_t)	The elliptocyte content of the transfused blood (E)	The haematocrit value for the transfused blood (R_t)	Recipient's elliptocyte content after transfusion obtained by extrapolation (C_0)
IV_2	78.0	280	44.0	27.6	1.78
IV_8	21.8	165	26.1	35.8	2.74
V_2	23.7	170	27.0	39.5	2.80
V_3	31.1	280	27.0	39.5	3.24
VI_3	4.6	100	19.5	38.6	3.64
VI_4	7.2	110	19.5	38.6	4.46
VII_1	5.09	40	44.0	32.7	4.35
VII_2	4.18	38	44.0	32.7	5.61
VII_3	9.53	38	44.0	32.7	2.61
VII_4	8.6	80	44.0	32.7	5.33
VII_5	9.13	100	44.0	32.7	5.63
$VIII_6$	8.29	70	27.3	40.5	3.10
IX_2	10.1	90	27.3	40.4	2.80
IX_3	18.9	150	27.3	40.4	3.05
IX_4	10.32	125	27.3	40.4	5.15
X_1	11.17	100	27.3	41.3	3.11
X_3	8.95	108	27.3	41.5	5.29
X_4	12.19	100	27.3	41.5	2.72
XI_2	33.6	250	23.2	38.6	2.40
XII_1	—	475	27.3	35.0	3.39

corpuscle weight. Table 23 gives the value for the amount of blood injected, its haematocrit value and C_0.

Calculations of the blood corpuscle weight.

The author thus carried out determinations of the corpuscle weight in 19 patients, most of whom were between about 1 and 12 years old (2 were adults). The elliptocyte count at time 0 was obtained by extrapolation from the experimental curve so that C_0 represented the elliptocyte count as found in the previous chapter by smoothing the experimental results with an exponential curve. C_0 may therefore be considered as a more reliable figure than values obtained by single observations made immediately after the transfusion. The first value

Table 24. Absolute and relative weight of red blood corpuscles in 19 experimental subjects.

Number	Age	Weight kg	Total weight of red blood corpuscles	
			in gram	in per cent of body weight
IV_2	32 years	78.00	1977.0	2.53
IV_8	6 »	21.80	545.0	2.50
V_2	9 » 6 months	23.70	628.2	2.65
V_3	12 »	31.10	877.1	2.82
VI_3	9 months	4.60	181.2	3.94
VI_4	2 years 2 months	7.20	154.2	2.14
VII_1	8 months	5.09	128.4	2.52
VII_2	6 »	4.18	91.8	2.20
VII_3	1 year 3 months	9.53	212.4	2.23
VII_4	1 » 3 »	8.60	204.6	2.38
VII_5	1 » 9 »	9.13	240.2	2.63
$VIII_6$	2 years 5 months	8.29	239.3	2.89
IX_2	3 » 8 »	10.10	343.1	3.40
IX_3	3 » 8 »	18.90	520.0	2.75
IX_4	2 »	10.32	234.8	2.28
X_1	1 year 10 months	11.17	346.5	3.10
X_3	1 » 1 month	8.95	201.2	2.25
X_4	4 years	12.19	404.4	3.32
XI_2	12 »	33.60	904.1	2.69
			Mean of all cases	2.70

used was taken at least 15 minutes after the completion of the transfusion. After this time one may assume that mixing is more or less complete. (This, however, is not so important in the present instance as the value being used is obtained by extrapolation from many values taken at later times.)

The corpuscle volume was calculated from the formula $V_r = V_e \cdot R_e \frac{(E - C_0)}{C_0}$, where V_e = the volume of blood injected in ml, R_e = the haematocrit value of the injected blood and E = the percentage elliptocyte count of the elliptocytic donor.

Table 24 shows the results obtained.

It is seen from the table 24 that *the weight of the blood corpuscles* (specific gravity = 1.08) as a percentage of the body weight *is*

Table 25. Total weight of red corpuscles expressed as % of the body weight determined by the use of elliptocytes and by two other methods.

$M \pm \varepsilon(M)$ = mean ± standard error of the mean.
σ = standard deviation.

Method	Weight kg	No. of cases	$M \pm \varepsilon(M)$	σ	σ in % of M
Radioactive P (Hevesy et al. 1944)	54—72	22	3.60 ± 0.09	0.417	11.6
Carbon monoxide (Hevesy et al. 1944)	54—72	19	4.42 ± 0.15	0.653	14.8
Elliptocytes (Hedenstedt)	5—78	19	2.70 ± 0.11	0.47	17.5

2.70 ± 0.11 %, which is less than commonly supposed.[1] The standard deviation of the values is 0.47 and is thus about the same as that obtained by Hevesy et al. with radioactive P, table 25. However, these workers have obtained much higher values of the blood corpuscle weight. An elucidation of the reasons for the discrepancies is thus necessary and the values obtained will be discussed. Hevesy et al. carried out simultaneous determinations of the weight of the corpuscles on the same patients using radioactive P and CO.

Comparison of different methods.

The table 25 shows that there is *a significant difference between the value for the weight of red blood corpuscles as obtained by Hevesy's method and that of the present author.* The value for the blood corpuscles obtained by the use of P_{32} is about 30 % higher than that obtained using elliptocytes. The fact that determinations using CO give such high values may be explained by some of the CO being taken up by the muscle haemoglobin.

Table 26 shows the results of some corpuscle weight determinations by Nylin using P_{32} on 5 normal cases. It is seen that even here the mean (3.3 %) is larger than that obtained by the present author.

[1] It is naturally possible to determine the *blood volume* as well when the haematocrit value for the recipient's blood would be included in the formula. The blood volume in ml as a percentage of the body weight is in this case, 7.42 ± 0.30.

Table. 26. Weight of red corpuscles determined by means of radioactive P (P_{32}) in 6 adults (from G. Nylin 1945).

Time after injection	Number of tests	Weight kg	Total weight of red corpuscles	
			g	in % of body weight
50—90 sec.	10	65	2390	3.68
15—20 min.	7	47	1789	3.80
3— 8 »	4	69	2073	3.00
3—15 »	15	75	2076	2.77
2— 9 »	16	63	1460	2.32
3—10 »	12	49	2200	4.48

It is surprising that the present author obtained a lower value for the blood corpuscle weight using his method than that which was obtained by the use of radioactive P. It would rather have been expected that the elliptocyte method would have yielded a higher value for the corpuscle weight if the blood in the depots had been significant in amount as, during these relatively long experiments the elliptocytes ought to have become mixed with the depot blood. As there is a systematic discrepancy between the results obtained by the two methods, the author has tried to find an explanation for it.

a) *Elliptocyte method.* If we take it that the elliptocyte method gives too low values for the corpuscle weight we have to assume that the elliptocyte concentration found is too high, i. e. the value for C_0 obtained by extrapolation is too high. The control calculations on case XII_1 described in the previous chapter show, however, that the same value is obtained for C_0 whether the first value is used or whether it is excluded on the suspicion that it may be too high owing to imperfect mixing. The author has convinced himself by calculations of the whole material that it is comparatively immaterial whether or not one includes the first value on blood taken 15 minutes after the completion of the transfusion. The sources of error discussed in chapter 11 with reference to the great scatter of the figures for the half-life must also be borne in mind in the present connection, although it seems to the author that one can hardly explain the great difference between the results obtained by the two methods on these lines.

b) Regarding *the method using* P_{32} there is also the possibility that this method may give too high a value for the corpuscle weight.

When trying to find a way whereby one might at least proceed towards an experimental solution of this discrepancy between the values for the corpuscle volume the author hit on the idea of using *"doubly labelled" corpuscles*. Thus the author had the corpuscles of an elliptocytic labelled with radioactive P so as to have the opportunity of seeing whether the dilution of elliptocytes and P_{32} coincided or whether they followed a different course. If the radioactive P does not leave the corpuscles during the course of the experiment the plot for the elliptocytes and that for the radioactivity should coincide within the limits of the experimental error and the results obtained for the blood corpuscle weight should be the same. The two methods would thus check one another. However, the reason for conducting this experiment was that there is a significant difference between the values for the corpuscle weight obtained by the two methods so it would be expected that, in this experiment, the concentration of the elliptocytes would be relatively higher than that of the radioactive P. This might be due to the corpuscles in elliptocytic blood loosing some of their radioactivity at the beginning of the experiment.

Experiments with "double labelling".

The author obtained radioactive P for this experiment through the kind help of Docent Örström, Stockholm.

Some days before bleeding the elliptocytic received a suitable intravenous dose of phosphate containing radioactive P. The radioactivity of the blood was then recorded at intervals until it reached a constant value which was about the third day after the injection. About $^1/_2$ litre of blood was then withdrawn from the elliptocytic whose blood now contained a suitable quantity of radioactive P. 4 experiments were carried out, 3 using washed corpuscles and 1 using whole blood. The corpuscles were washed 3 times in sterile Ringer's solution for 20 minutes at a time. The plasma which has been washed away was finally replaced with sterile Ringer's solution. Samples were taken before transfusion for determining the radioactivity of both the plasma and corpuscles in the donor's blood and its haematocrit value and elliptocyte count. The amount of blood used and the technique of administration were the same as in ordinary blood transfusions. Samples were withdrawn at frequent intervals after the completion of the transfusion, and even in one case during the course of it. The same samples were used for the determination of the ellipto-

Table 27. Table giving the values obtained simultaneously for the elliptocyte count and specific radioactivity (P_{32}) of both corpuscles and plasma after transfusion of elliptocytic blood labelled with radioactive P in 3 children. Donor's elliptocyte content = 27.3 and his specific radioactivity of corpuscles = 3.06.

Case X_1				Case X_3				Case X_4			
	Whole Blood				Washed corpuscles				Washed corpuscles		
Hours after end of transfusion	Elliptocytes %	P_{32} corpuscles	P_{32} plasma	Hours after end of transfusion	Elliptocytes %	P_{32} corpuscles	P_{32} plasma	Hours after end of transfusion	Elliptocytes %	P_{32} corpuscles	P_{32} plasma
0.25	3.36	0.392	0.211	0.25	5.17	—	0.056	0.25	2.87	0.254	0.015
1.00	3.64	0.280	0.149	1.08	5.88	0.458	0.072	0.75	-	—	0
4.08	2.57	0.249	0.101	4.00	5.61	0.385	0.055	4.08	3.19	0.231	0.022
6.00	3.15	0.219	0.070	6.08	5.62	0.358	0.064	5.25	2.95	0.209	0.089
18.58	2.82	0.165	0.062	18.25	5.40	0.207	0.086	17.75	2.70	0.104	0.039
45.58	2.80	0.121	0.015	42.25	5.22	0.110	0.254	41.75	2.29	0.097	0
69.58	2.61	0.0708	0.0396	66.33	5.20	0.044	0.167	65.83	2.28	0.022	0
93.58	3.11	0.093	0.0158	90.42	3.62	0.041	0.001	89.75	1.98	0.082	0
117.58	2.55	0.017	0.0516	114.42	3.56	0.026	0.000	113.92	1.82	0.017	0

Table 28. Table giving the values obtained simultaneously for the elliptocyte count and specific radioactivity (P_{32}) of both corpuscles and plasma after a transfusion of elliptocytic blood labelled with radioactive P in an adult (case XII_1). Donor's elliptocyte content = 27.3 per cent and his specific radioactivity of corpuscles = 1.51. The red corpuscles were washed before used.

Time during and after transfusion	Elliptocytes, %	P_{32}, corpuscles	P_{32}, plasma
1 min. after beginning of transfusion	0.11	0.0025	0.019
5 » » » » »	0.51	0.0312	0.082
8 » » » » »	1.37	0.0930	0.009
10 » » » » »	1.95	0.0856	0
0 » » end » »	1.95	0.0856	0.014
5 » » » » »	2.69	0.181	—
10 » » » » »	—	0.183	0.040
15 » » » » »	4.86	0.181	0.03
20 » » » » »	3.97	0.181	0.03
35 » » » » »	3.22	—	—
50 » » » » »	3.18	0.179	0.03
65 » » » » »	3.07	0.182	0.027
365 » » » » »	3.45	0.172	0.0102
19 hours » » » »	3.27	0.080	0.073

cyte count and the corpuscles were separated from the plasma by centrifugation immediately after the withdrawal of the sample. The radioactivity of both corpuscles and plasma was then determined. The radioactivity was expressed as specific activity, i.e. the number of impulses per unit weight of total phosphate present (see p. 71).

Using the formula already given for the determination of the corpuscle volume with elliptocytes, one can substitute the specific activity of the donor's blood and that of the recipient after transfusion for the values E and C_0. One thus gets pairs of expressions for the value of the corpuscle weight at different times after the transfusion.

Experiments with double labelling were carried out on 4 cases of which one was an adult and the rest were under 12 years old. Table 27 and 28 shows the specific activities of the corpuscles and plasma and the elliptocyte count.

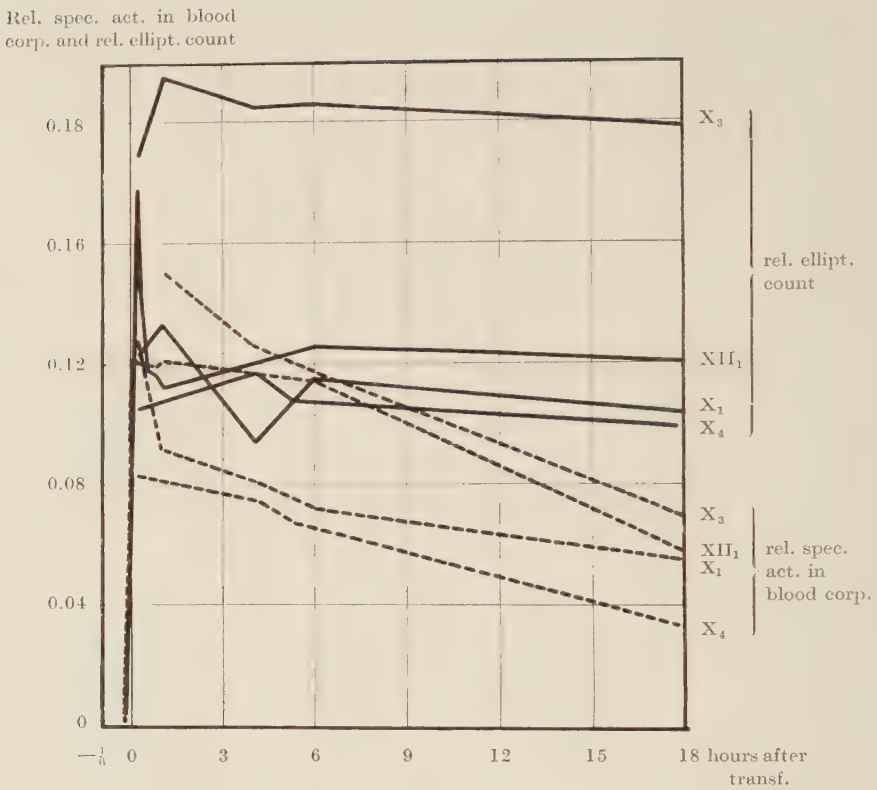


Fig. 15. Comparison between the relative activity in red blood corpuscles and relative elliptocyte content during and after intravenous transfusion in three children (X_1, X_3, X_4) and one adult (XII_1).

Relative specific activity of blood corpuscles is the ratio between specific activity of the recipient's blood corpuscles and specific activity of the donors's blood corpuscles. Relative elliptocyte content is the ratio between per cent elliptocyte content in the recipient and per cent elliptocyte content in the donor.

In order to obtain comparable values for the specific activity and elliptocyte count, the values obtained for these after transfusion were divided by the specific activity and elliptocyte count of the donor, which are given in the table headings. Thus the relative elliptocyte count and relative activity in 4 cases may be compared in fig. 15.

a. *Comparison of values obtained by elliptocyte counts and radioactivity estimations using double labelling* (fig. 15).

3 of the plots (X_1, X_3, X_4) show clearly that when at least an hour has elapsed after the conclusion of the transfusion the elliptocytes

Table 29. Comparison between the values obtained for red blood corpuscle weight by the simultaneous use of radioactive P (P_{32}) and elliptocyte counts at different times after the end of transfusions in 3 children.

C_0 = value of elliptocyte content in % at time 0 obtained by extrapolation.

Time after end of transfusion	Corpuscle weight in g		Corpuscle weight in % of body weight		
	P_{32}	Elliptocytes	P_{32}	Elliptocytes	Difference in % of elliptocytes
Case X_1					
15 min.	304	318	2.72	2.85	— 4.6
1 hour	443	290	3.97	2.60	+52.7
4 hours	504	429	4.51	3.84	+17.4
(C_0)	—	346.5	—	3.10	—
Case X_3					
15 min.....	—	207	—	2.31	—
1 hour	274	176	3.06	1.97	+55.3
4 hours	335	187	3.74	2.09	+78.9
(C_0)	—	201.2	—	2.25	—
Case X_4					
15 min.....	495	381	4.06	3.13	+29.7
4 hours	548	338	4.50	2.77	+62.5
(C_0)	—	404.4	—	3.32	—

have lost a considerable amount of their activity. Now these experiments were made on children in whom it may be supposed that the metabolism is different from that in adults owing to growth etc.

The fourth plot (XII_1) was obtained from an experiment on an adult and shows a difference in that on the whole the plots for the elliptocyte count and radioactivity follow one another. There is, however, a distinct discrepancy 15—20 minutes after the end of the transfusion but the author cannot account for its occurrence in this single case.

b. *Comparison between the values for blood corpuscle weight obtained by double labelling.*

In order to find out how the blood corpuscle weight behaves

when determined simultaneously by both methods the author has calculated the values for the blood corpuscle weight obtained by the simultaneous use of radioactive P and elliptocytes at different times after the transfusion (table 29).

The table shows that the values for the corpuscle weight in children (X_1, X_3, X_4) within one hour after end of the transfusion are definitely higher when determined by radioactive P except for the value obtained 15 minutes after the end of the transfusion in case X_1 where the values obtained by both methods were about the same. It is only to be expected that the difference will increase with the length of time from the end of the transfusion as the radioactivity of the corpuscles gradually decreases. In case XII_1 the position is different in that the values obtained by both methods correspond pretty well except for that obtained 15—20 minutes after the end of the transfusion where the discrepancy was the same as in the children (table 30).

Table 30. Comparison between the values obtained for red corpuscle weight by the simultaneous use of radioactive P (P_{32}) and elliptocyte count in an adult (XII_1).

Time after end of transfusion	Corpuscular weight in kg		Difference in % of elliptocytes
	P_{32}	Elliptocytes	
5 min.	1.318	1.641	−19.7
10 »	1.303	—	—
15 »	1.318	0.828	+59.2
20 »	1.318	1.054	+25.3
35 »	—	1.341	—
50 »	1.332	1.360	− 2.1
65 »	1.310	1.415	− 7.4
365 »	1.397	1.239	+12.8
19 hours	3.205	1.317	+143.4

This difference between the values for blood corpuscle weight obtained by using double labelling is in agreement with the significant difference obtained by comparison of Hevesy's et al. method and that of the present author (see table 25).

Discussion of results.

As was pointed out above, these experiments with double labelling were only designed to find the reason for the discrepancy between the values for the blood corpuscle weight obtained by the use of elliptocytes or radioactive P. It would be possible to check the one method against the other and to study such deviations as might occur, by carrying out a larger series of experiments with doubly labelled corpuscles. It is only thus that one can get definite information as to which of the methods may require correction. However this experiment confirms the suspicion that under the experimental conditions described above the dilution of corpuscles labelled with radioactive P may be greater than that of the elliptocytes. This is because *the radioactive isotope may leave the corpuscles more freely than was formerly believed during the period immediately after the transfusion.*

THIRTEENTH CHAPTER.

Transperitoneal Resorption of Red Blood Corpuscles.

On the Time Course of the Transperitoneal Resorption of Red Blood Corpuscles (Model Experiments).

There are many ways by which it may be imagined that the physiological resorption of corpuscular elements from the peritoneal cavity could occur. The problem is probably very complicated from the kinetic point of view. However, certain purely hypothetical assumptions have been made as to the theoretical nature of the process with a view to finding out what "might be expected". It might be possible to obtain some indication as to the *drainage* of the corpuscles, its *rate* and the *fragility* of the corpuscles by comparing the values obtained on the basis of these assumptions.

In working this problem out theoretically the author has tried, as far as possible, to avoid a purely mathematical solution and has tried instead to visualise the process by experiments on models where these could be performed. It was found possible to use a mechanical model which "automatically" carried out the necessary mathematical operations.

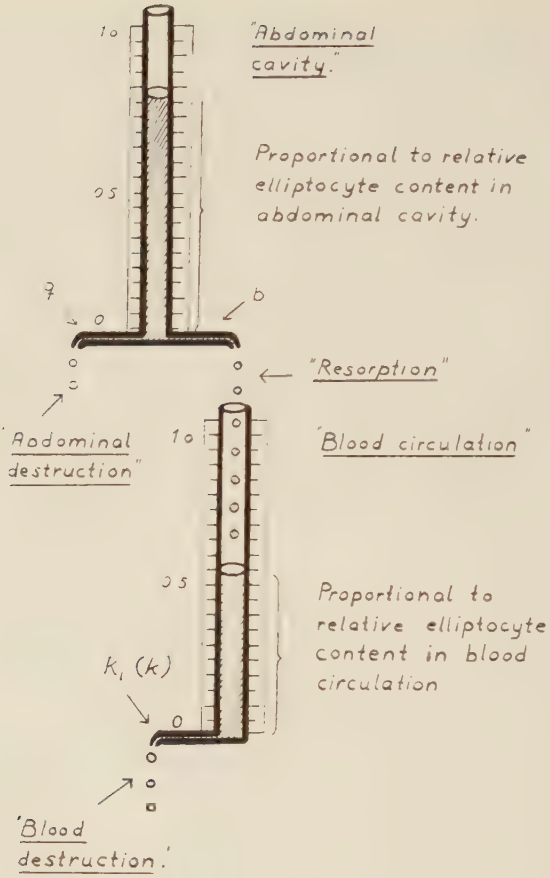


Fig. 16. Scheme of model apparatus (Teorell 1937), showing the features of transperitoneal resorption of elliptocytes (the exponential case).

For demonstration of the intravenous case only the lower tube is used. For demonstration of the uniform resorption, see text.

The fundamental principle of the model (which was constructed by T. Teorell (1937) and later modified), is that it consists of 2 glass cylinders one above the other joined by capillary tubes so that liquid can run from the upper to the lower cylinder. Both cylinders also have capillary outflow tubes. If one allows the liquid to flow from the upper cylinder into the lower one can convince oneself, by taking readings on a scale, that the outflow from the upper tube occurs according to an exponential curve, i. e. with a *rate proportional to the prevailing height of the liquid column*, which may represent the "concentration" of the blood corpuscles in the abdominal cavity resp. the blood circulation. The same applies to outflow from the other capillary outflow tube in the upper cylinder and to the outflow from the lower cylinder. Fig. 16 shows a diagram of the model and elucidates its function.

The object of the model is to imitate the course of the processes which actually take place. The upper cylinder can be taken to represent the *abdominal cavity* and the lower one *the vascular system*. One of the two capillary tubes leading from the upper cylinder represents the loss caused by *destruction of the corpuscles in the abdominal cavity* indicated by q and the other the actual process of resorption the intensity of which is indicated by b. If the upper cylinder is raised to a considerable height above its capillary outflow into the lower one, the inflow into the lower tube will occur at an approximately *uniform* rate. The capillary tube which forms the outflow for the lower cylinder is indicated by k_i (k), which is the constant for the *destruction of corpuscles in the blood* (see fig. 16). Different constants for the different contributory processes may be obtained by altering the calibre of the capillary tubes. (For the sake of simplicity the author has used the same resorption constant b and the same q and k_i for all his experiments).

By the use of this simple model one can obtain purely hypothetical curves for the expected behaviour of the process being studied. The curves referred to in the following discussion were all obtained with Teorell's model. The model cannot, however, imitate a uniform absorption process with abdominal destruction, but such a system can be rather easily understood by a little mental reasoning (fig. 17).

A. Constant rate of resorption (the uniform case).

Assume *a constant rate for the flow of lymph from the abdominal cavity to the blood* which forms the basis for all drainage. Assume furthermore that the corpuscles injected into the peritoneal cavity are from the beginning uniformly distributed in the peritoneal fluid and are thus transported uniformly into the blood vessels (i.e. the same number of corpuscles is transported from the abdominal cavity per unit period of time). The curve showing the number of corpuscles in the abdominal cavity at varying times in its simplest form (i.e. in absence of abdominal destruction) must be a straight line (case A: 1. fig. 17). The time required for the transport of all the corpuscles into the blood vessels is the "resorption time" (t_r). When resorption is complete the destruction of blood corpuscles in the vessels only occurs in the normal way which is still, as was previously assumed, according to an exponential law (see chapter 11). The maximum on the "blood"-curve which is affected by both processes, thus indicates the completion of resorption after which only intravascular destruction of corpuscles takes place (at least for sufficiently high resorption rates).

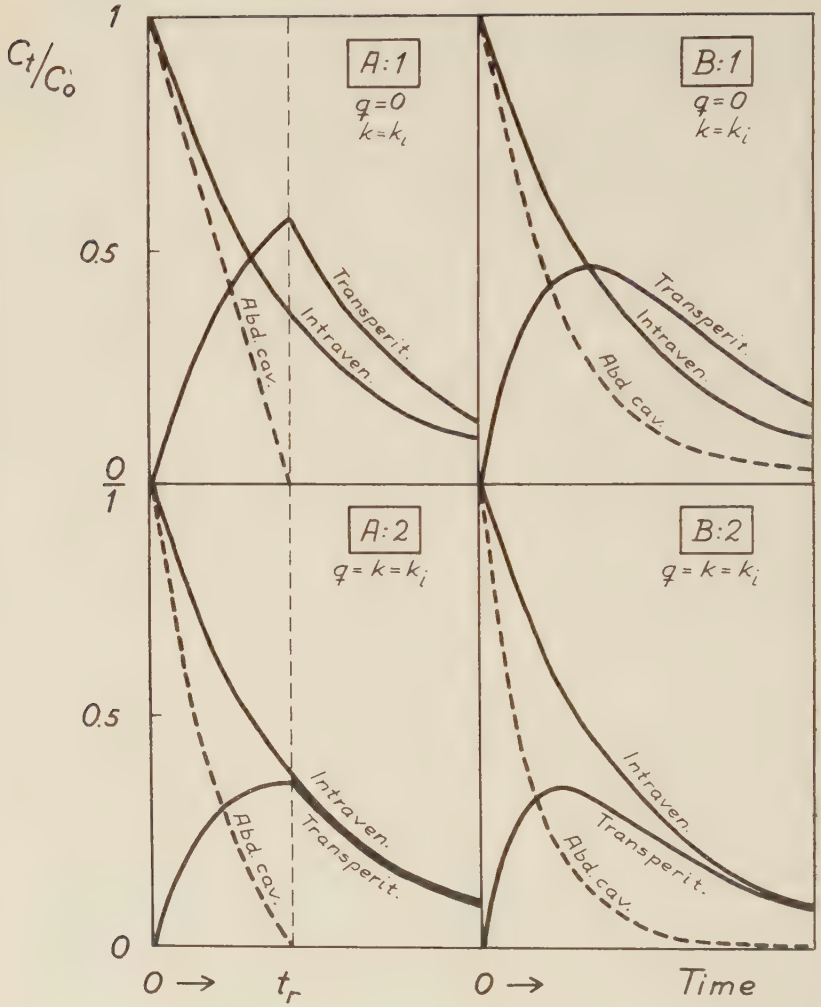


Fig. 17. Theoretical curves for the *uniform* resorption cases (A:1 and A:2) and the *exponential* resorption cases (B:1 and B:2) obtained by aid of the model apparatus shown in fig. 16.

Case A:2 mathematically calculated for the same condition as A:1. t_r = the time for complete resorption.

If uniform resorption is combined with destruction or not in the abdominal cavity and the blood vessels one may imagine the occurrence of the following cases:

1. *The corpuscles in the abdominal cavity are destroyed at the same rate as if they had been injected directly into the blood-stream* (A: 2). It is seen that, according to this assumption, the curve for the *falling off* of the number of corpuscles in the blood will coincide with the later part of the curve obtained if the blood had been given intravenously instead of intraperitoneally. It would be a matter of comparative indifference whether the corpuscles were lying in the peritoneal cavity or in the vessels if they were destroyed at the same rate. Thus it is clear that the maximum of the transperitoneal curve would (at the *higher* resorption rates) lie on the corresponding intravenous curve. If q = the destruction constant for corpuscles in the abdominal cavity, k = the destruction constant for the resorbed corpuscles and k_i = that for corpuscles injected intravenously, then, in the special case postulated $q = k = k_i$. As was mentioned above, the model cannot be used to demonstrate the course of resorption in this case. However it is possible with a formula deduced by Teorell (see p. 118) to construct the corresponding curves (case A: 2, fig. 17). If the resorption times were of unequal length in different individuals, owing to the lymph flowing at different rates, the maxima would be relatively widely scattered and there might be some difficulty in distinguishing such a curve from the one which would be obtained if resorption followed an exponential course.

2. *The corpuscles in the abdominal cavity are not destroyed* (A: 1). In this case (where there is no loss of corpuscles in the abdominal cavity), the number of corpuscles absorbed per unit period of time will be greater than in the previous case where $q = k = k_i$. In this case $q = 0$ and $k = k_i$. This assumption should therefore mean that higher maxima will be found on the curves as there are a larger number of corpuscles "alive" at the time when it occurs than in the previous case. The maximum must thus lie *above* the curve obtained by intravenous injection. If resorption is really uniform and the corpuscles in the abdominal cavity are distributed in the same volume of peritoneal fluid regardless of the number of corpuscles injected, then the time of occurrence of the maximum should be constant for any given rate of lymph flow, and independent of the number of corpuscles injected. It is seen from fig. 17 case A: 1 that the expected curve corresponds well with that obtained by experiments with the model.

3. *Transition forms.*

a. *Corpuscles in the abdominal cavity are destroyed faster than those in the blood* ($q > k_i$, $k = k_i$). It is clear that in this case the curve for transperitoneal resorption will lie below that for intravenous injection.

b. *Corpuscles in the abdominal cavity are only destroyed to a moderate extent* ($k_i > q > 0$, $k = k_i$). The maximum will then lie above that for the intravenous curve and will be intermediate between A: 1 and A: 2.

c. *The corpuscles are only destroyed to a moderate extent in the abdominal cavity but after resorption they have a different destruction constant from that they would have had if they had been injected intravenously.* It might, for instance, be thought that the weakest forms are destroyed in the abdominal cavity and only the more resistant forms are resorbed in the intact state and that they would therefore have a greater mean resistance than corpuscles injected intravenously, $k > q > 0$, $k \neq k_i$. This is the most general case. The deviations in the rate of destruction of the corpuscles can best be observed by comparing the calculated destruction constants for blood injected intravenously with the falling part of the curve obtained after intraperitoneal transfusion. (In all cases described under A the falling part of this curve is purely exponential, having the form $C_t = C_0 \cdot e^{-kt}$. In its logarithmic form this is strictly parallel with the curve obtained after intravenous injection only in cases where the destruction constant for corpuscles after resorption is the same as that after intravenous injection).

B. Exponential resorption (the exponential case).

The process may best be imagined by thinking of the corpuscles as lying in a mass somewhere within the abdominal cavity, and supposing that the corpuscles are removed from the surface or edge of the mass (i.e. the chance of the constant flow of lymph into the blood vessels containing a large proportion of corpuscles is higher in the earlier stages of resorption, when the mass of blood is larger than in the later stages).

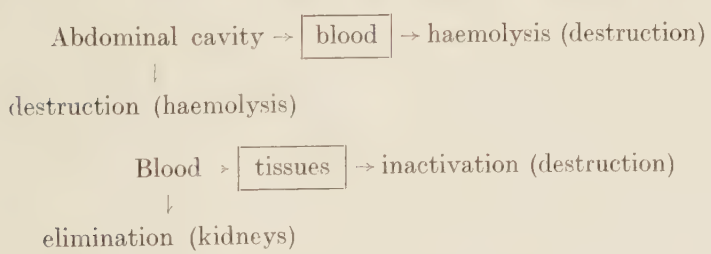
It may also be imagined that, as in cases A, the corpuscles are uniformly distributed throughout the fluid in the abdominal cavity but that the corpuscles have to pass through a sieve-like membrane (stomata in the diaphragm etc.) during resorption. The chance of many corpuscles passing through the sieve is greater in the earlier stages of resorption, when the number of corpuscles is large, than in the later stages.

It is impossible to calculate the exact form of this function for the rate of resorption. One might assume, as the simplest practical approximation, that *the fraction of the total amount of corpuscles (intact and hæmolyzed inclusive) resorbed per unit period of time (rate of resorption) is proportional to the number of corpuscles remaining*

in the abdominal cavity. As may easily be seen the curve for the accumulation of corpuscles in the blood will have a rising portion and a descending portion in this case too. The latter portion should approach nearer and nearer to a simple exponential curve of the type obtained after intravenous transfusion. (In the later stages of resorption, after the maximum has been reached, the accession of corpuscles from the abdominal cavity is very small, see fig. 17 (cases B) which shows curves obtained with the model representing the emptying of the abdominal cavity under these conditions. An "exponential" resorption gives a curve which is more flattened at the maximum than one obtained for uniform resorption (cf. A: 1). It follows that very little resorption occurs after the maximum point on the accumulation curve has been reached). Obviously the rate of resorption = the rate of destruction in the blood at the maximum of the curve.

Like uniform resorption, exponential resorption can be imagined to have varying forms, depending on the destruction of the corpuscles in the abdominal cavity and the vessels.

1. *The corpuscles in the abdominal cavity are destroyed in the same way as if they circulated in the blood* ($q = k = k_i$). The shape of the curve obtained for this case (B: 2) is fairly complicated. An analogy may be made with the resorption of a drug administered intravenously and eliminated from the blood by the kidneys during the period while it is being inactivated or destroyed in the tissues by an exponential process. The corpuscles resorbed into the blood are considered to represent the concentration of the drug in the tissues. The following diagram, taken from Teorell, illustrates this point.



It should be possible to illustrate the process by reference to Teorell's tissue-curves (1937). The conclusions drawn, namely that the time at which the maximum is reached is independent of the

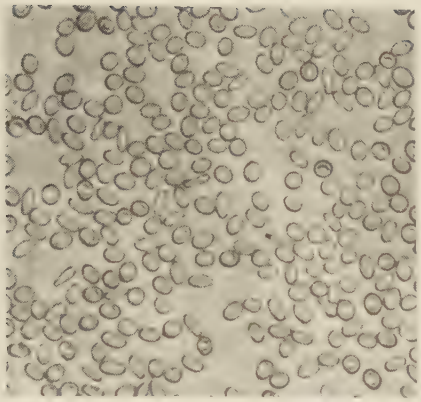
dose and that its height is proportional to the dose should thus hold approximately for this case too. There is, however, one important difference between the process at present being discussed and the kinetic process covered by Teorell's theory, namely that this theory is based on the assumption that the transfer of the drug from the blood to the tissues depends on the difference in concentration and is reversible and not, as in the mechanism proposed in this case for the resorption of corpuscles, on the number of corpuscles and transported one way only. Teorell's formulae can (see foot-note p. 121), however, be easily modified to include also the seconditions. In any case the rising part and the maximum of the curve will lie below that obtained by intravenous transfusion. In Fig. 17, case B: 2 this type of curve is illustrated by the use of Teorell's model.

2. *The corpuscles are not destroyed in the abdominal cavity.* ($q = 0$, $k = k_i$). Making the same assumptions as before, one should get a curve with a higher maximum than that in the above case (B: 1). Actually it can be demonstrated that the maximum value lies on the intravenous curve. Hence the falling part of the curve which represents both continued resorption and destruction in the blood, should lie above that for the curve obtained for intravenous transfusion. As resorption gradually ceases the same curve will eventually be in character identical with the intravenous curve with the destruction constant as found for blood transfused intravenously. If the rate of resorption varies from case to case the maximum will occur earlier where the rate of resorption is faster. The maximum should occur at about the same time with different doses if the rate of resorption remains constant. The curve obtained by an experiment with the model under these conditions is given in fig. 17, case B: 1.

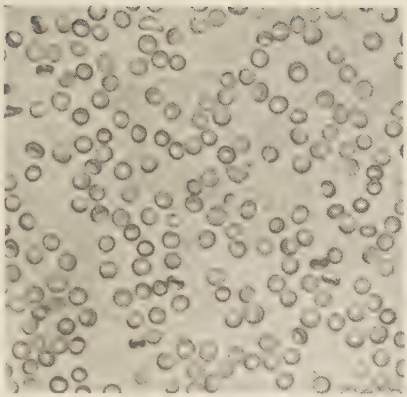
Experimental Investigations with Elliptocytes.

The author has carried out 28 intraperitoneal transfusions of elliptocyte blood into children between the ages of a few days and 12 years. The amount of blood transfused was, on the average 13 % of the blood volume of the recipient. (Blood volume = 7.4 % of the body weight, see p. 97).

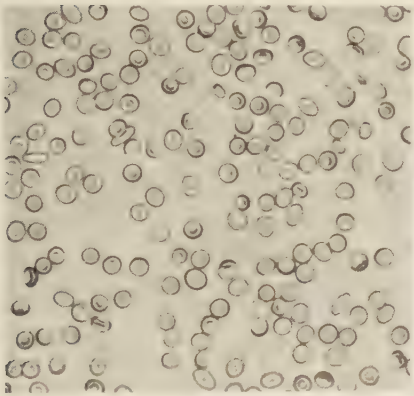
Table 31 shows the observed values for the various cases. All the cases except V_6 were included in the following calculations.



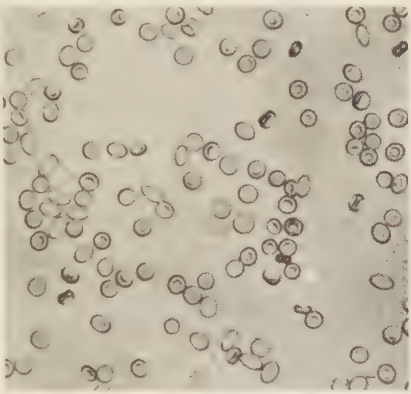
Blood from an elliptocytic (the donor). 27.3 % elliptocytes.



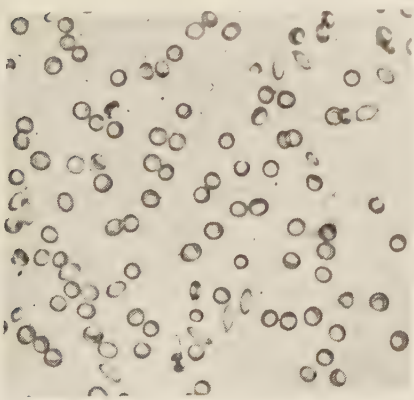
Blood from the experimental subject before transfusion (the recipient) 0.44 % elliptocytes.



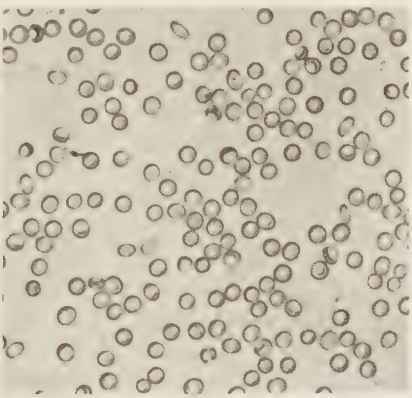
5 days efter transfusion 5.60 % elliptocytes.



8 days after transfusion 9.8 % elliptocytes.



16 days after transfusion 8.45 % elliptocytes.



45 days after transfusion 1.04 % elliptocytes.

Fig. 18. Elliptocyte count in the blood stream before and after intraperitoneal transfusion of elliptocytic blood to a normal subject (case V_6).

It is seen that in case V_6 the elliptocyte concentration in the blood was found to be considerably higher than was expected, assuming the value for the corpuscle volume[1] obtained by the author (see p. 97) and thence calculating the value which would have been obtained if the blood had been injected intravenously. The child in question probably had a considerably smaller blood corpuscle volume than the average. Fig. 18 shows photographically the elliptocyte count in this child before and on several occasions after the transfusion.

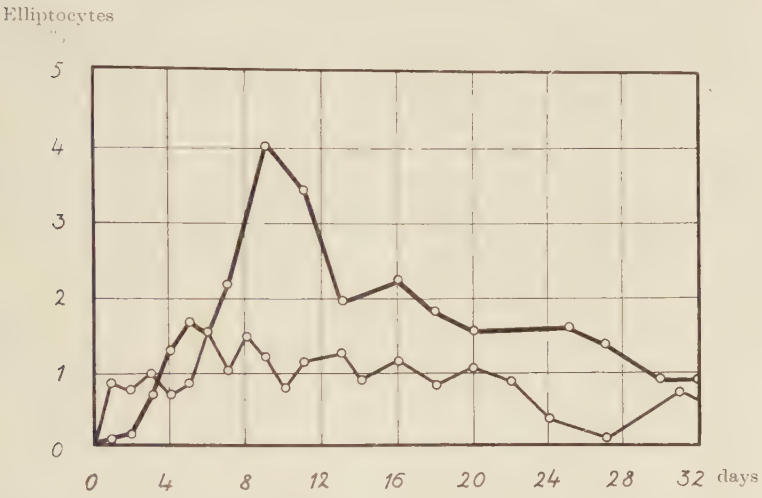


Fig. 19. One of the most regular (III_{10}) and one of the most irregular (II_4) of the transperitoneal curves.

Fig. 19 shows one of the more regular and one of the more irregular cases in order to give an idea of the course of the individual curves.

Corrections and transformations.

Correction for error due to "mutual dilution". As was pointed out previously in chapter 11 the original elliptocyte content of the recipient was simply subtracted from the values obtained after the transfusion. Actually, the original content of the elliptocytes belonging to the recipient, which is usually very small before the transfusion, should diminish during the period the corpuscles are resorbed from the peritoneal cavity acting in a "diluting" way. Accordingly the relative elliptocyte count ought to be lowest at the time of maximal

[1] The value for the blood corpuscle volume 2.5 % is obtained by dividing the blood corpuscle weight 2.7 % with the spec. grav. 1.08.

Table 31. Percentage elliptocyte content in different norma

Day after transfusion	Case												
	I_2	I_3	I_4	I_5	I_6	I_7	I_8	II_1	II_2	II_4	III_1	III_2	III_3
0	0.48	0.76	0.4	0.75	0.97	0.11	0.16	0.16	0.39	0.74	0.44	0.30	0.27
1					0.81			0.85	0.46	1.59	1.48	0.31	0.57
2	1.40	1.00	0.78	1.33	0.56	0.14	0.34	0.83	0.51	1.52	1.31	1.00	2.37
3	2.71	2.27	2.26	2.20	1.11	0.39	0.86	1.10	0.95	1.74	1.13	1.24	2.55
4	3.00	3.51	3.17	3.20	1.62	1.09	1.11	1.14	1.32	1.46	2.34	2.21	2.71
5	3.48	5.22	4.05	5.75	2.03	1.17	1.06	1.34	2.83	1.60	1.54	2.94	1.92
6	4.92	5.13	5.18	5.46	1.81	1.55		1.32	2.16	2.28	1.98	2.93	1.84
7	5.08		5.02	5.32	2.07	1.47	1.30	1.77	2.99	1.77	1.62	3.33	
8	4.88	6.20	4.40	6.12	1.62	2.59	1.49	1.94	2.40	2.24			
9	3.78	5.01	4.91	4.33	2.32	2.02	1.27	2.07	2.26	1.97	2.52	2.54	
10								1.88	2.17	1.54			
11	4.94	6.67	5.17	3.82	2.93	2.22	1.59	1.90	3.25	1.90	2.53	2.25	
12	4.75	6.34	5.39		2.13	2.20	1.31						
13					1.87					1.99	2.22	2.24	
14	4.72	5.54	4.26	4.24		1.35	2.21	1.56	3.36	1.63			
15													
16	4.00		3.37	4.20		1.45	1.54	1.62	2.65	1.89	2.13	1.39	
17													
18	2.71	4.93	3.26	3.38	1.90	1.49	1.98	1.10	2.75	1.57	1.40	1.66	
19													
20								1.84	2.85	1.82	0.54	0.97	
21	3.44	5.31	2.58	3.49	1.50	0.93							
22								1.50	2.75	1.61			
23	2.80	2.67	3.04	2.86	1.48	1.34	1.19						
24								0.96	1.73	1.11			
25	3.00	4.67	2.85	3.20	1.41	1.12	0.88				0.88	0.82	
26									1.95				
27								0.89		0.62	0.83	1.05	
28		4.59		3.24	1.39	1.47	1.12						
29													
30	3.13	5.36			1.45	0.75	0.99				0.89	0.57	
31								1.10	1.73	1.50			
32	1.31	5.24			1.39	0.76	0.66				0.76	0.47	
33													
34								1.17	1.29	1.12			
35	1.46	3.10			1.41	0.72	0.75						

subjects after *intraperitoneal* transfusion of elliptocytic blood.

Case														
III_4	III_5	III_6	III_7	III_8	III_9	III_{10}	IV_3	IV_4	IV_6	IV_7	V_4	V_6	IX_8	X_2
0.50	0.15	0	0.41	0.53	0.11	0.18	0.16	0.37	0.12	2.35	1.29	0.44	0.17	0.32
1.23	0.38	0.06	0.13	0.63	1.01	0.34	0.26	0.44	0.21	2.04	1.60	0.32	0.46	2.81
1.03	0.94	1.88	0.29	0.88	1.03	0.38	0.23	0.60	0.92	3.89	1.78	1.08	0.43	2.91
1.11	2.36	3.17	1.00	1.13	1.70	0.84	0.46	0.87	1.13	2.72	2.35	1.44	0.37	3.30
1.10	1.75	3.68	0.86	1.18	1.53	1.49	1.38	1.53	1.28	3.17	3.56	1.74	1.02	3.45
2.56	2.40	3.35	1.56	2.61	1.68	1.88		1.54	2.38	3.27	3.13	5.60	1.32	
2.78	2.89	2.72	1.37	3.38	1.65	1.73	3.05	1.55	3.13	3.56	3.94			3.44
1.72	3.92	3.56	1.40	2.37	1.08	2.39	2.10	1.27	3.10	4.92	3.42	7.88	1.35	3.67
											3.49	9.80		3.90
2.27	1.68	2.92	1.06	3.46	1.65	4.21	2.15	1.77	2.65	3.12	3.00	9.21		3.95
													1.81	3.94
1.81	1.80	2.20	1.33	2.47	1.77	3.60	1.71	1.75	2.15	3.08	2.81	9.30		3.03
													1.38	
1.97	1.62	1.83	0.89	2.72	1.56	2.13	2.39	1.36	2.29	2.84				
											2.84	7.40	1.60	
														2.28
1.79	1.64	1.58	1.00	2.23	1.44	2.41	2.09	1.30	1.62	3.30	2.25	8.45		
													0.88	2.26
1.59	1.42	1.29	1.00	0.98	1.66	2.00	1.95	0.87	1.75	3.49	1.97	7.49		
													0.84	2.59
1.29	1.54	1.04	0.76	1.23	1.24	1.76	1.12		1.77	2.06				
													0.75	
														1.04
1.62	0.85	0.84	0.97	1.25	1.35	1.78							0.57	
														1.81
1.43	1.04	0.73	0.59	1.09	1.09	1.57								1.04
													0.60	
														1.09
1.25	1.12	0.69	0.50	1.03	0.70	1.06								
														1.00
0.72	0.99	0.64	0.29	1.02	0.96	1.05								
													0.34	0.53
													0.38	

resorption. It should then rise slowly to attain its original value when the resorbed corpuscles have been destroyed in the blood. Nor are corrections required in the cases in which the transfusion was given intraperitoneally.

The construction of the mean curve from the whole material which should serve as a basis for further calculations necessitates certain transformations of the figures. This is partly because the various experimental subjects received different amounts of blood with different elliptocyte counts and haematocrit values and partly because they themselves had different blood volumes and haematocrit values.

These differences may conveniently be corrected by using the value for the blood corpuscle volume [see foot-note p. 113] to calculate the value for C_0 using the same "*mixing formula*" which was previously used for calculation of the corpuscle volume (see p. 96). In this formula the amount of blood transfused, its elliptocyte count and haematocrit value are all known. (C_0 was the value for the elliptocyte count which would have been obtained if the blood had been transfused directly into the veins). A value can thus be obtained for C_0 in every case, and this can then be used for correction of the observed values.

All experimental values in a series are then divided by the corresponding C_0. *The ratio C_t C_0 is a relative measure of the elliptocyte content which makes possible a comparison between the different cases* in spite of variations in volumes, red blood cell counts etc. and mode of administration. The C_t C_0 is accordingly expressed in terms of the maximal value (i.e. initial value at the time 0) of an intravenous transfusion given under identical conditions. The values of C_t/C_0 ought to vary between 0—1.

This transformation is carried out both on the intraperitoneal and the intravenous cases. (The intravenous material consists of 20 cases which are the same as those described in chapter 11). When the whole material, including the cases in which the transfusion was given intraperitoneally and those in which it was given intravenously, had thus been made comparable, a mean curve was constructed for the various observations at different periods after the transfusion.

Quantitative treatment of the experimental results.

The smoothing procedures applied to the entire experimental material which are described below serve two purposes, a) to enable

presentation of "*representative*" *curves* of the intravenous and transperitoneal transfusion results and b) to yield *functional data* which could be employed in an attempt to elucidate the quantitative *nature of the transperitoneal blood corpuscle resorption.*

We will accordingly first describe the numerical smoothing procedures and take up the discussion of the significance of the smoothing results later (on p. 123).

Smoothing procedures.

In chapter 11 and the first part of the present chapter we have developed certain views as to the nature of the time course of the elliptocyte count following intravenous and intraperitoneal blood transfusions. The intravenous cases were treated as yielding exponential "die away" curves according to the simple function $C_t = C_0 \cdot e^{-kt}$. The transperitoneal resorption on the other hand was referred to one of two alternatives: 1) the "*uniform*" or "*linear*" resorption case where the blood cell transport (abdominal cavity → blood) occurs at a constant rate (= "cases A"); 2) the "*exponential*" case where the cell transport occurs at a diminishing rate (= "cases B"). These cases were clarified by appropriate model experiments and illustrated graphically (cf. p. 110 and fig. 17).

When we now want to perform a numerical treatment of our results it is necessary to fall back on a few mathematical formulas which describe quantitatively what the model demonstrations have shown us in a more qualitative manner.

We will start with I: *Exponential smoothing of the intravenous transfusion results* and continue with II: *Smoothing of the transperitoneal transfusion results* postulating first a) a *uniform* resorption and then b) an *exponential* resorption.

I. *Cases in which the transfusion was given intravenously.* The values employed in the smoothing were averages of observations obtained on a varying number of individuals who were investigated at equal times after the transfusions.

The smoothing was performed on the plot of the averages by means of the exponential function previously used and using the method of least squares on the logarithmic curve. The observed mean values for the first 31 days were included in the calculation and weighed in accordance with the number of individual cases represented in each mean value.

The smoothing function thus obtained was:

$$\frac{C_t}{C_0} = 0.956 \cdot e^{-0.0476\,t}.$$

k_i is 0.048. The half-life was thus 14.6 days. The observed and smoothed values are given in table 32. The observed values seem to be quite well smoothed by this function (see fig. 20).

II. *Cases in which the transfusion was given intraperitoneally.* The experimental values employed in the smoothing were averages obtained from observations on a varying number of individuals at one and the same interval after the transfusion. In smoothing these values it is therefore necessary to take into account the number of cases from which the average was obtained.

a) *Assuming uniform resorption:*

Here the process consists of two portions one more complicated taking place until the total amount of blood cells in the abdominal cavity is resorbed (the "rising" part of the curve) and thereafter a simple exponential decay:

The rising part of the curve for the cases in which the transfusion was given intraperitoneally (up to and including the 8th day) were smoothed by the use of the function:[1]

$$\frac{C_t}{C_0} = \frac{1}{t_r} \cdot t \cdot e^{-kt}.$$

using the method of least squares after appropriate transformations. (t_r = the time required for complete resorption of the corpuscles from the peritoneal cavity into the blood circulation, in other words $1/t_r$ is the rate of the (uniform) resorption). The equation for the curve was found to be:

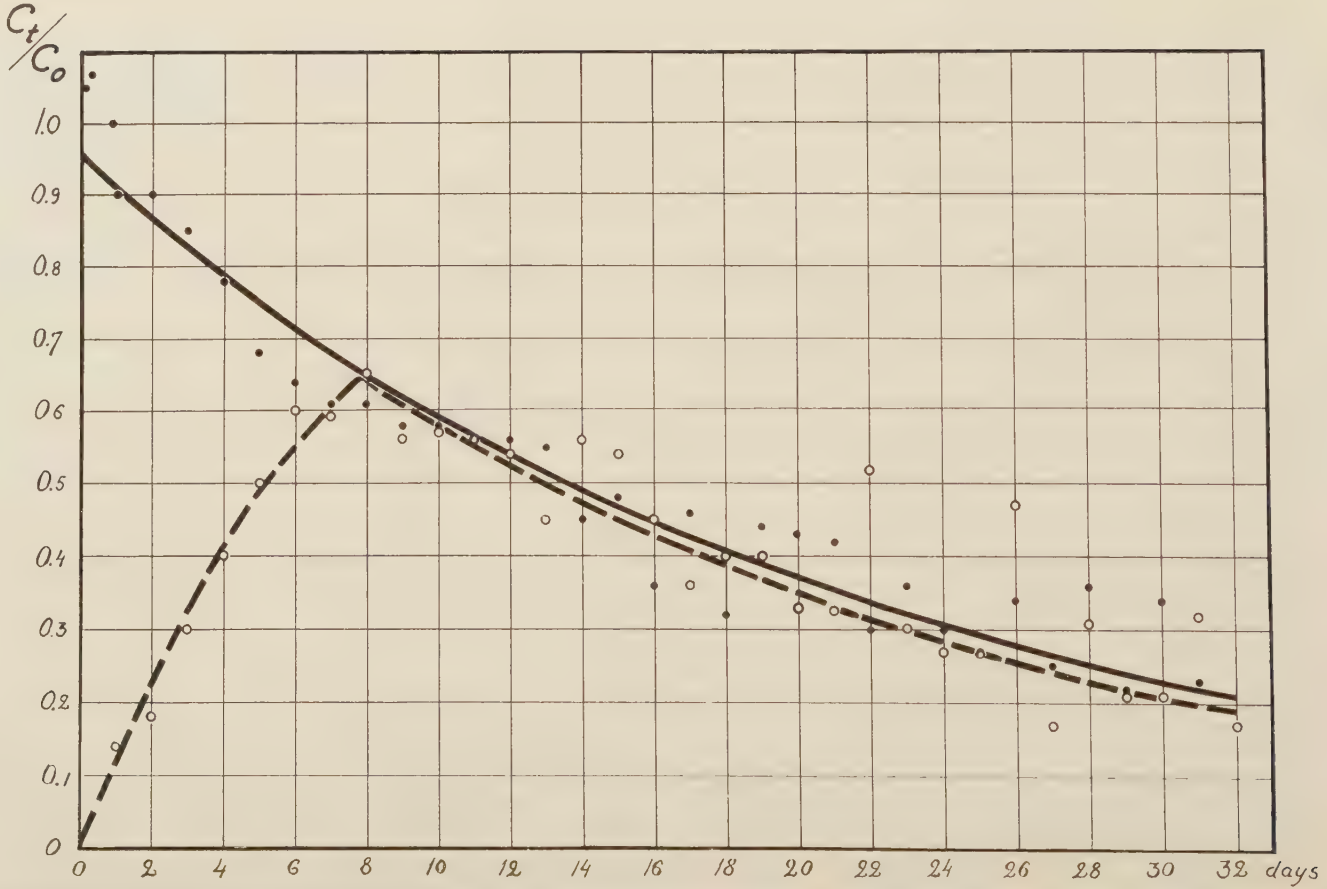
$$\frac{C_t}{C_0} = 0.123\ t \cdot e^{-0.0497\,t}.$$

The values smoothed according to this equation and the observed values are given in table 32. The values appear to be well smoothed

[1] The formula for this function which represents the case in which resorption occurs *uniformly* and in which the *destruction of corpuscles occurs at the same rate in the abdominal cavity as in the blood circulation* ($q = k$), was obtained by the author from Teorell, who worked it out in connection with other resorption formulas (see the works of Teorell). Cf. model experiments on p. 108.

Table 32. The observed and smoothed average values of ellipto cyte content after intravenous and intraperitoneal transfusion.

Time after transf. (days)	Intravenous transfusion			Intraperitoneal transfusion		
	No. of cases	Mean of C_t/C_0 observed	smoothed	No. of cases	Mean of C_t/C_0 observed	smoothed
0	—	—	0.956	—	—	0 (0.958)
0.17	13	1.05	0.948	—	—	— (0.950)
0.30	6	1.07	0.942	—	—	— (0.944)
0.70	8	1.00	0.925	—	—	— (0.925)
0.90	14	0.90	0.916	—	—	— (0.916)
1	—	—	0.912	21	0.14	0.117 (0.911)
2	20	0.90	0.851	26	0.18	0.222 (0.866)
3	20	0.85	0.829	27	0.30	0.317 (0.824)
4	19	0.78	0.790	27	0.40	0.402 (0.783)
5	14	0.68	0.753	25	0.50	0,479 (0.745)
6	18	0.64	0.718	25	0.60	0.546 (0.708
7	17	0.61	0.685	25	0.59	0.606 (0.673)
8	15	0.61	0.653	12	0.65	0.659 (0.640)
9	14	0.58	0.623	25	0.56	0.609
10	7	0.58	0.594	5	0.57	0.579
11	7	0.56	0.566	25	0.56	0.550
12	9	0.56	0.540	7	0.54	0.523
13	5	0.55	0.515	15	0.45	0.498
14	12	0.45	0.491	11	0.56	0.473
15	4	0.48	0.468	1	0.54	0.450
16	9	0.36	0.446	22	0.45	0.428
17	5	0.46	0.426	2	0.37	0.407
18	2	0.32	0.406	24	0.40	0.387
19	10	0.44	0.387	2	0.40	0.368
20	1	0.43	0.369	15	0.33	0.350
21	5	0.42	0.352	7	0.33	0.332
22	4	0.30	0.335	3	0.52	0.316
23	7	0.36	0.320	8	0.30	0.301
24	4	0.30	0.305	3	0.27	0.286
25	2	0.27	0.291	17	0.27	0.272
26	3	0.34	0.277	2	0.47	0.258
27	5	0.25	0.264	12	0.17	0.246
28	2	0.37	0.252	6	0.31	0.234
29	2	0.22	0.240	1	0.21	0.222
30	1	0.34	0.229	14	0.21	0.211
31	1	0.23	0.219	4	0.32	0.201
32	—	—	0.208	14	0.17	0.191


C_t/C_o
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 days

in this way (see diagram, fig. 20). Attempts to use smoothing formulas where $k + q$ were less successful.

The smoothing of *the descending part* of the curve for the cases in which the transfusion was given transperitoneally was carried out in the same way as in the cases where it was given intravenously using an exponential function as described in chapter 11. The smoothing was carried out on a part of the curve from the 8th day onwards. The method of least squares has been applied to the function in its logarithmic form; the observed values were thus included as weighed means. The smoothing function obtained was

$$\frac{C_t}{C_0} = 0.958 \cdot e^{-0.0504\,t}.$$

The values obtained by smoothing according to this equation and those of the actual observations are given in table 32 ($k = 0.050$). The observations appear to be well smoothed by these means. The half-life was 13.7 days (see fig. 20).

b) *Assuming "exponential" resorption:*

The model demonstration described previously (p. 110) has shown the general features of an exponential resorption in presence and absence of blood corpuscle destruction also in the abdominal cavity taking place simultaneously with the resorption to the blood. The corresponding mathematical formula[1] derived from Teorell's work

[1] The formula is $\frac{C_t}{C_0} = \frac{b}{k - (b + q)}\,(e^{-(b+q)t} - e^{-kt})$ where b is a "resorption constant", k and q retain their previous significance.

Fig. 20. Comparison between *intravenous* and *intraperitoneal* transfusion experiments (a »uniform» resorption course is postulated.)

Observed mean values and smoothed curves (filled circles and continuous line = i. v. case, open circles and dashed line = i. p. case).

The curves fit the equations:

a. intravenous curve: $\frac{C_t}{C_0} = 0.956 \cdot e^{-0.048\,t}$

b. transperitoneal curve:

1. rising branch ($t < t_r$): $\frac{C_t}{C_0} = 0.123\,t \cdot e^{-0.050\,t}$

2. descending branch ($t > t_r$): $\frac{C_t}{C_0} = 0.958 \cdot e^{-0.050\,t}$

t_r = the time in days for total resorption of the red blood corpuscles.

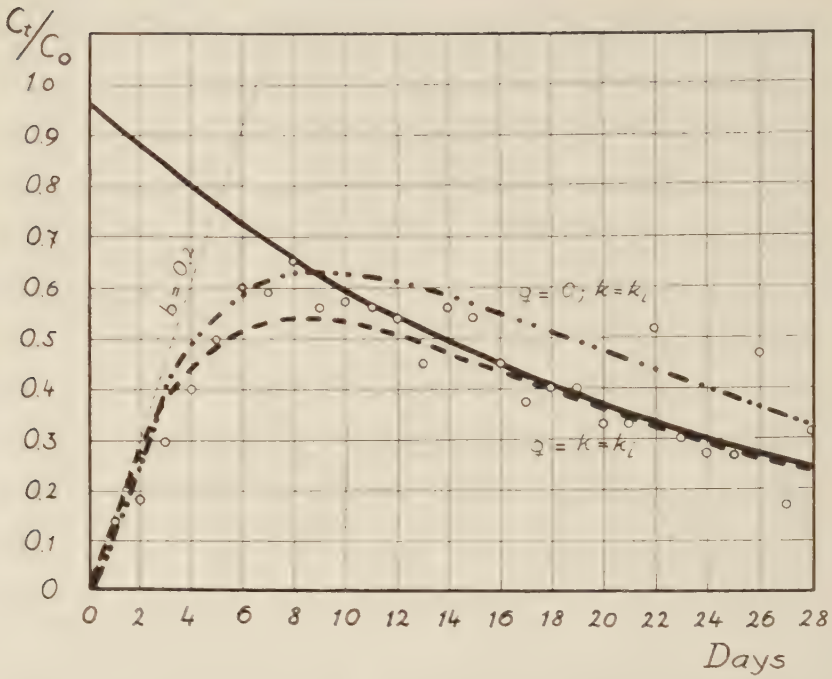


Fig. 21. A comparison between the observed *transperitoneal values* (open circles) and some calculated *exponential* resorption curves, according to formula in foot-note p. 121.

The observed intravenous curve is also plotted (as a continuous line). $k_i = 0.050$.

(1937) has been fitted to our experimental results by the method of trial and error until a satisfactory agreement was found.

The result is illustrated in fig. 21. The value of b was 0.20 and two curves are plotted, one with $k = q = 0.0497$, i.e. about the same value as found for intravenous transfusion and another where k still is 0.0497 but $q = 0$ (no abdominal blood destruction). Neither alternative as to the magnitude of q gives a satisfactory fit, although one perhaps has the impression that the observed points can fall between the two curves. There seems, however, to be a significant discrepancy in the early periods: the calculated curves rise much steeper than the curve corresponding to the experimental points. Other values of the parameters have been tried but no improvement was obtained. Actually the uniform smoothing previously preformed gives better fit to the experimental observations than any of the exponential trials.

In conclusion it might be stated that the "uniform" smoothing presented in the previous paragraph is more successful than the "exponential" smoothing.

Discussion of Previous Calculations.

Linear or exponential resorption? The results of the various alternatives for curve smoothing obtained in the foregoing section point towards the probability of a more or less uniform resorption rate of blood cells from the peritoneal cavity (cf. the excellent results of procedure II a) above). This would be in conformity with a picture of *a continuous lymphatic drainage through the peritoneal cavity* which "washes out" its possible content of corpuscular particles in an astonishingly complete manner.

Rate of resorption — the speed of the lymphatic drainage: The rate of resorption ($1/t_r$) is obtained either from the inverse value of the time of the maximum[1] of the curve ($t_r = 8.0$ days, $1/t_r = 0.125$) or from the tangent at the origin to the rising part of the curve which yields almost the same value: $\frac{1}{t_r} = 0.123$.

In other words this would mean that *a "foreign", corpuscular content introduced in the peritoneum is completely resorbed to the blood circulation after about 8 days, or, provided no destruction occurs on the route of resorption, about 12 % of the foreign elements is drained per day.* Strictly speaking such a conclusion ought to be limitated to particles of similar size and character as those of the elliptocytes.

As regards the elliptocytes or common red corpuscles the simultaneous destruction during the process of resorption will cause a gradually declining value of the amount of intact cells actually entering the blood circulation (cf. also fig. 20 and relevant discussion on p. 124).

One might perhaps generalise these conclusions: *it may be conceivable that also other corpuscular elements as bacteria in cases of peritonitis, peritoneal exudates etc. might have a "clearance" of a similar magnitude.* Thus it may be possible that these results with elliptocytes can throw some light on certain surgical problems of interest.

[1] This is true at least for values of, at the time t_r, $\frac{C_t}{C} > 0.37$ according to Teorell's formula.

It should be pointed out that it is impossible with the data at hand to tell how large the *real* lymphatic drainage is. The results later described on the temporary fall of the haemoglobin value might indicate that the drainage of liquid (for instance plasma) takes place at a higher rate than that of suspended particles (cf. discussion p. 129).

Destruction of the corpuscles. The course of resorption is complicated by the fact that, as has previously been mentioned, account has to be taken of the fact that a certain amount of destruction of the corpuscles occurs within the abdominal cavity during the progress of the resorption. If we assume what is probably true, namely that resorption occurs at a uniform rate, it is easily seen from the results obtained by the smoothing procedure that we are dealing with a case which is generally similar to that which was described as A: 2 in the theoretical section. In this especial case it was assumed that $q = k = k_i$ and the maximum of the curve will lie on the curve for intravenous injection of the elliptocytic blood. This seems also to be the case in the material observed. The curve for the intraperitoneal injections falls at a comparatively constant rate after reaching its maximum on the 8th day. If the corpuscles were destroyed to a greater or lesser extent in the paths of resorption than in the blood vessels this would be revealed by a lower or higher value respectively for the elliptocyte count in the circulating blood at the time of completion of resorption (about the 8th day) than would have been the case on the corresponding day after the intravenous transfusion of the elliptocytic blood. As has been stated the maximum elliptocyte count is reached on the 8th day and C_t/C_0 is then 0.65 when calculated as the average for 12 cases. On the 7th and 9th days the values (based on 25 cases) are 0.59 and 0.56 respectively. The value obtained on the 8th day after intravenous transfusion is 0.61 and the values for the adjacent days are fairly similar (0.61 and 0.58) (see table 32). This fact is a *strong indication that the destruction of blood corpuscles is about equal both in the abdominal cavity and in the blood circulation* assuming that resorption occurs at a uniform rate. It also suggests that, as mentioned above, resorption is complete by the 8th day. The values for k and k_i obtained by the smoothing processes agree surprisingly well ($k = 0.050$ and $k_i = 0.048$).

Rate of emptying of the abdominal cavity.

The author has previously spoken of the resorption of blood from the peritoneal cavity into the blood vessels on several occasions. The corpuscles do not however pass directly through the peritoneum into the circulating blood but have first to traverse the lymph glands and lymph vessels in the mediastinum (cf. literature, chapter 5). Some authors (including Efskind and P. F. Hahn and their co-workers) have also pointed out that the lymph vessels and glands in the mediastinum are stuffed with particles or corpuscles after intraperitoneal injections. It would therefore be more correct in principle to speak of *the paths of resorption*, including both the peritoneum and the mediastinum. It has, however, been more convenient in previous references to speak schematically of resorption direct from the abdominal cavity into the circulating blood. Nevertheless it is of some interest to find out how long the corpuscles remain in the abdominal cavity and mediastinum. The author has not done a large series of experiments in this connection. Two of the cases in the present material are, however, of interest in this respect.

First, the case IX_7, mentioned on p. 129 in which about half of the amount of blood transfused was resorbed within 24 hours. This experiment indicates that the abdominal cavity itself empties much faster than the mediastinum. In the other case (IX_6) the author performed a similar experiment injecting normal blood into an acranial infant, the injection amounting to about 12 % of the blood volume. At autopsy 24 hours later the whole of the blood had been resorbed from the abdominal cavity. This is in agreement with what previous authors have found in animal experiments in which the abdominal cavity was opened at various intervals after intraperitoneal injection of blood. They found that the contents of the abdominal cavity were emptied very quickly.

These two experiments show that *the blood corpuscles during resorption may spend a considerable time within the lymphatic system of the mediastinum.*

Histological investigation of the paths of resorption.

Comprehensive histological investigations of various sorts on animals have been described in the literature. They all lead to the same conclusion as to the path of resorption of various sorts of corpuscular particles from the abdominal cavity (cf. chapter 5). It thus appears that there is fair unanimity as to the existence of at

least functional stomata and lymph lacunae under the peritoneal epithelium even in the higher animals. Allen's photograph (fig. 22) gives a beautiful demonstration of a nucleated frog's blood corpuscle in a mouse making its way through the roof of such a lacuna.

The author has not carried out any comprehensive histological investigations into the paths of resorption in man but has largely relied on analogies with animal experiments. In one case (IX_7), however, the author had the opportunity of observing that the *conditions appear to be the same in man as in animals.*

In this case (see p. 129) autopsy revealed that a thin layer of blood was adherent to the diaphragmatic peritoneum. At the autopsy which took place immediately after death, great care was taken to avoid contamination of the peritoneum with blood, but the blood which was already in the abdominal cavity was removed carefully with a spatula without disturbing the surface of the diaphragm. Suitable pieces were removed from parts of the diaphragm where the layer of blood could be seen to be lying, and were embedded in paraffin. Serial sections were then cut and stained with haematoxylin and eosin. The results are seen in fig. 23 and 24.

Lymph lacunae are seen which appear to be of the same sort as those described in animals. The pictures give a definite impression that these lacunae might have a free connection with the peritoneal cavity (stomata). One of the lacunae contains blood corpuscles which appear to be elliptical. This, however, must be considered with some reservations as deformation of the red corpuscles can easily occur in such histological preparations. This experiment gives good support to the original opinion put forward by von Recklinghausen, namely *that corpuscular elements leave the peritoneal cavity through temporary openings also in man.*

Haemoglobin concentration and red cell count associated with transperitoneal resorption of red blood corpuscles.

Nobody has obviously been able to demonstrate the resorption of intact living red blood corpuscles from the abdominal cavity by means of measurements of haemoglobin percentages and red cell counts in conjunction with intraperitoneal blood transfusions. This is probably because there is in fact no increase in the haemoglobin or number of red cells in the circulating blood during the first few days after the injection. The present author found rather a temporary decrease of these values during the first part of the process of

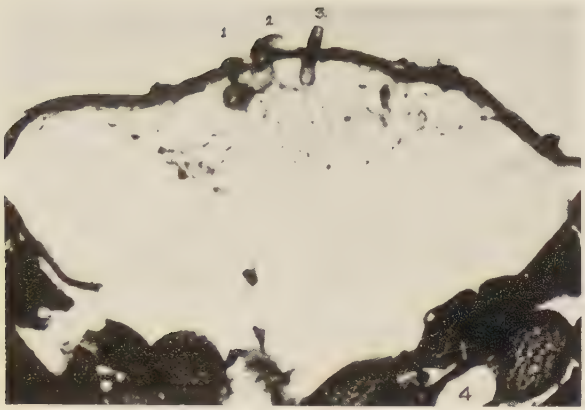
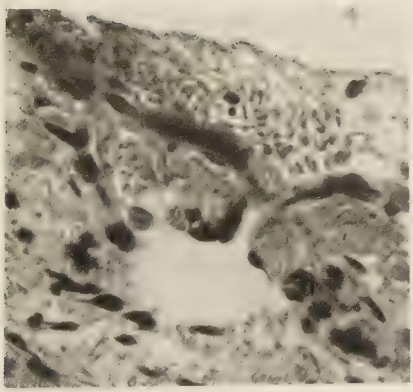


Fig. 22. Peritoneal resorption of frog erythrocytes in mice. 1, 2 and 3 showing erythrocytes on their way through the roof of a lacuna. From the paper of Allen 1936.



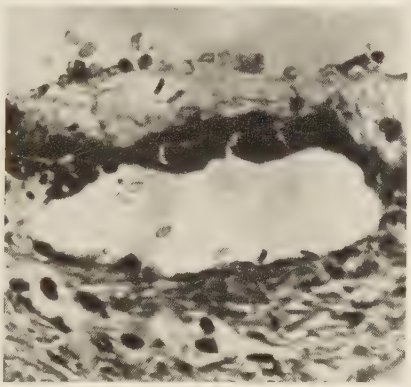


Fig. 23. A lacuna from the peritoneal surface of the diapragm in a human child with communication to the peritoneal cavity, probably a stoma.

Fig. 24. A lacuna from the above mentioned case, in which an intraperitoneal injection of elliptocytic blood was made. Notice on the peritoneal surface a loose clot of fibrin and red blood corpuscles and in the lacuna 4 red corpuscles, the form of which suggests elliptocytes.

resorption (see also Opitz and Metis chapter 5, p. 51 and others) This is probably the reason why previous authors have doubted whether intact blood corpuscles really are transferred from the peritoneal cavity into the circulation.

The author has carried out haemoglobin determinations and blood counts on a large section of the material during and after the period of resorption.

In the compilation of the results the author has not included children aged less than 14 days in order to avoid, to some extent, errors caused by the physiological decrease in these values during the earliest days of life (cf. Vahlquist, 1941, and others). Furthermore only children who received a fairly large amount of blood (on the average 13 % of the blood volume) were included, and, of course, only those on which the determinations were carried out with sufficient regularity during the first week after the transfusion.

Table 33 shows the average value for the percentage of haemoglobin and the red cell count. The blood values are expressed in relative terms, i.e. as a percentage of the value for the percentage of haemoglobin or red cell count which the patient had immediately before the transfusion.

Table 33. Relative values of haemoglobin and red blood corpuscles at different days (in per cent of the values before transfusion) after intraperitoneal transfusion.

Day	Red blood corpuscles		Haemoglobin		
	No. of cases	Mean	No. of cases	Mean	Mean of 13 cases*
0	16	100	15	100	100
1	12	96	14	95	95
2	16	92	15	92	91
3	16	94	15	91	92
4	16	97	14	94	94
5	15	99	14	98	98
6	11	97	14	96	96
7	15	99	14	98	97
8	10	91	9	96	—
9	14	105	10	95	—
10	8	99	12	100	
11	14	102	14	97	—

* These cases were observed every day.

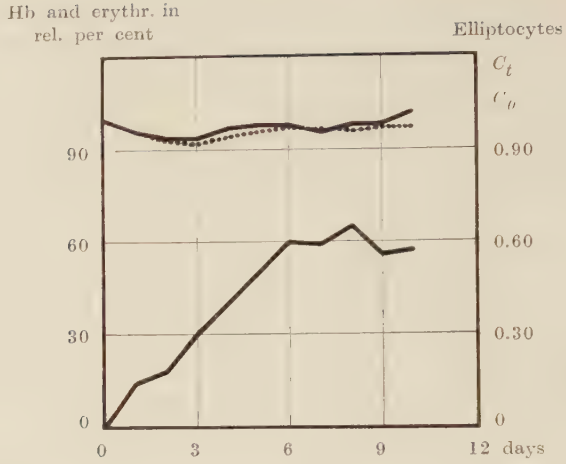


Fig. 25. The relative average values of hæmoglobin and red blood corpuscles after intraperitoneal transfusion.

The upper continuous line = red blood corpuscles } Smoothed with successive means
The dotted line = hæmoglobin }
The lower continuous line = transperitoneal curve.

Table 33 and fig. 25 show that *a slight fall in both haemoglobin concentration and red cell count occurs during the 1st—3rd days after the transfusion* and that these values then rise again to their level before the transfusion. The decreases in the values for the haemoglobin concentration and red cell count are about 9 % and 8 % respectively, and this corresponds approximately to the value calculated on the assumption that the circulating blood is diluted by the plasma from the injected blood. The values are higher than would have been expected, but, remembering the unreliability of blood-volume determinations, (see chapter 12) no better agreement could have been expected.

In order to get a clearer idea of what occurs the author injected plasma alone intraperitoneally into a child (case X_5). The amount injected was calculated to be about 17 % of the child's blood volume. The haematocrit values for the child were followed before, and for some days after the transfusion. They are expressed as a percentage of the values obtained before transfusion. The following values were obtained,

Table 34.

Before	transfusion	=	100
1 day after	»	=	78
2 days »	»	=	88
3 » »	»	=	96

The figures show that in this case also there was *a decrease of about 22 % in the relative value of the red corpuscles 24 hours after the injection* with a gradual rise later. The decrease corresponds rather well to what was to be expected.

These three experiments all show that a decrease in haemoglobin concentration and red cell count may occur during the earlier stages of transperitoneal resorption when blood or plasma are injected intraperitoneally. The only plausible explanation for this is that the plasma is resorbed into the blood stream more rapidly than the corpuscles and dilutes the circulating blood temporarily until a sufficient proportion of corpuscles have been resorbed to restore the balance. There is fairly good quantitative agreement between the volume of blood injected and the dilution of the circulating blood.

The author carried out the following experiment in order to get a better insight into the concentration of the blood in the abdominal cavity.

An acranial infant (case IX_7) received 60 ml elliptocytic blood (about 32 % of the child's blood volume) barely 24 hours before its death. Autopsy revealed that about half of the blood had been resorbed. The haemoglobin concentration and red cell count of the elliptocytic blood were determined before the transfusion and on the blood which remained within the abdominal cavity. The elliptocyte count in the circulating blood had only risen by an insignificant amount during the short period while half of the blood had been resorbed from the abdominal cavity. This suggests that a large proportion of the blood is lodged in the lymphatics during resorption. It was further found that a considerable amount of haemoconcentration had taken place in the abdominal cavity, *the red cell count in the blood which remained in the abdominal cavity having risen from 3.8 to 5.5 million and the haemoglobin concentration from 80 to 105 %.*

This is a further confirmation for the circumstance that *the plasma is resorbed into the blood vessels faster than the corpuscles.*

Question of the formation of abdominal adhesions after intraperitoneal blood transfusion.

It was shown in the review of the literature that, for various reasons, opinions are divergent as to the danger of leaving blood in the abdominal cavity after operations or internal haemorrhages. The author has no personal experience in this matter. However, in order to clarify the matter the author has been in contact with the Sahlgren Hospital in Gothenburg and received information about a case which received repeated intraperitoneal injections of blood. Dr. Sydow supplied written communications and extracts from the case notes.

The case was that of a girl, barely one year old who was treated for aplastic anaemia by intraperitoneal injections of blood and subsequent splenectomy. The child had received not less than 10 intraperitoneal blood transfusions during the previous year, the first occurring 10 months and the last 21 days before the operation. The largest transfusion was of 140 cc of heparinised blood and the smallest 75 cc. The great majority were of 100 cc. On the whole the child tolerated the transfusions well. At the subsequent laparotomy for splenectomy the surgeon observed that *the peritoneum was smooth and shining without any adhesions or other changes.*

Summary of the Results of the Transperitoneal Resorption.

1. The corpuscles of elliptocytic blood are resorbed (without change in fragility etc.) from the abdominal cavity into the blood through the lymph vessels of the mediastinum, in all probability at a uniform rate.
2. Resorption of the injected blood takes about 8 days. About 12 % of a non-destructible, corpuscular content, coming from the peritoneal cavity, are resorbed every day to the circulating blood.
3. During and after the actual process of resorption the corpuscles are destroyed in the same way as though they had been injected intravenously, i. e. half of them "die" within 13—14 days.
4. The injected blood undergoes haemoconcentration in the abdominal cavity and the paths of resorption because the plasma is resorbed faster than the corpuscles.

5. The resorption of corpuscles from the abdominal cavity occurs more rapidly than that from the mediastinum.
6. Intraperitoneal adhesions need not occur even after repeated intraperitoneal injections of blood.
7. Histological investigations suggest that in man the corpuscles are resorbed through stomata into the subepithelial lymph lacunae in the diaphragm.

General Summary.

A *review of the literature* is given first. This shows that opinions are divided on many points. In the later part of the work experiments using elliptocytics are described. The whole work is based on 51 transfusions, mostly on children, using 10 elliptocytics. The results obtained are as follows:

1. In the first place an investigation was made into the degree of accuracy with which elliptocytes could be estimated quantitatively. It is found that estimation by eye gives satisfactory results and that the *percentual error of measurement amounts to rather less than 10* % in a count of elliptocytes and that it remains constant down to low levels i. e. about 1/2 %. The counts were made on photographs of diluted blood samples. Attempts to determine the ellipticity by measuring the axes of the elliptocytes were found to be too laborious and this method was therefore abandoned. It was found that the highest limit at which a cell was considered as elliptocyte when estimating by eye was an axial ratio of about 0.6.

2. A detailed comparison between elliptocytics and normal persons shows:

a) that normal persons show a low degree of elliptocytosis;

b) that the degree of elliptocytosis among elliptocytics varies somewhat from person to person. The difference between elliptocytics and normal persons is thus one of degree, but it is very pronounced so that there is no difficulty in distinguishing them. Repeated tests have shown that individual elliptocytics show a constant degree of elliptocytosis, and that *they do not manifest any symptoms of disease.*

3. The author has performed experiments counting 2 000–4 000 red blood corpuscles at intervals during the 30 days following intravenous transfusion. Furthermore one experiment was carried out counting 8 000 - 10 000 corpuscles in which the elliptocyte count was thus

more accurately determined. The *mixing time* for the elliptocytes after transfusion in the experiment, just described, amounted to at least 15 minutes. It appeared from this experiment that the corpuscles disappear approximately according to an exponential curve and that their *half-life* is 13.1 ± 0.9 days.

4. A new method has been devised for determining *the blood corpuscle weight* (*and blood volume*) in man using elliptocytes. The weight of the blood corpuscles is, on the average, 2.7 ± 0.1 % of the body weight. (Blood volume = 7.4 ± 0.3 %.) In spite of the fact that these determinations of the blood corpuscle amount should include the depot blood corpuscles, the values obtained for the blood corpuscle weight are significantly lower than those obtained by other authors using other methods such as labelling with radioactive P. The author introduced a method of "double labelling" in an attempt to find an explanation for these experimental discrepancies. For this purpose the corpuscles of an elliptocytic were labelled with radioactive P. By this means it is possible to check the one method against the other. The experiments were carried out on children and it was found that the isotope leaves the corpuscles to a greater extent than had previously been thought during the period immediately following the transfusion. In this way it was also found that calculations of the blood corpuscle amount based on the elliptocyte count gave lower values than those based on the estimation of radioactive P.

5. The author has studied the *resorption of blood corpuscles from the abdominal cavity into the blood stream* by performing intraperitoneal transfusions of elliptocytic blood on children and making repeated blood counts. A model constructed by Teorell was used in order to visualise the processes of resorption. Certain type curves were obtained by this means. Furthermore analogies were drawn from certain processes of distribution of drugs treated by Teorell and the formulae he deduced in this connection were used to construct curves on the basis of the observed data. From these experiments and calculations the author arrived at the following conclusions:

The resorption of corpuscular particles from the abdominal cavity into the blood stream is probably a process which takes place at a uniform rate. Blood corpuscles injected into the abdominal cavity

are completely resorbed to the blood circulation within 8 days (the rate of resorption is ca 12 % per day). During and after the period of resorption the corpuscles are destroyed in the abdominal cavity, lymph vessels and blood circulation at approximately the same rate as though they had been injected intravenously. This means, amongst other things, that the blood corpuscles do not undergo any significant alteration in vitality as a result of the process of transperitoneal resorption. No conclusion can be drawn from these experiments as to the mechanism causing the destruction of the corpuscles within the abdominal cavity as the biochemical and physical aspects of the subject are unknown and have not been the objects of research.

The author can also show that a haemoconcentration of the injected blood probably takes place in the abdominal cavity and the paths of resorption owing to the fact that plasma passes along the lymphatics more easily than corpuscles. Accordingly, there is a temporary dilution of the blood corpuscles in the circulation during the early stages of resorption. It appears that the corpuscles spend the greater part of the resorption time in the lymphatic pathways and that the abdominal cavity drains relatively quickly. Concerning the paths of resorption the author has performed histological investigations and come to the conclusion that in man, as in animals, the corpuscles pass through stomata into lymphatic lacunae lying under the peritoneal epithelium in diaphragm. The author finally shows that peritoneal adhesions need not develop even after repeated intraperitoneal blood transfusions.

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